THE TOTAL SYNTHESIS AND BIOLOGICAL EVALUATION OF BRYOSTATIN 1 AND ITS ANALOGUES

by

Yam Bahadur Poudel

A dissertation submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Chemistry

The University of Utah

May 2011

The University of Utah Graduate School

STATEMENT OF DISSERTATION APPROVAL

The dissertation of **Yam Bahadur Poudel**

has been approved by the following supervisory committee members:

Gary E. Keck	, Chair	**12/01/2010** Date Approved
Chris M. Ireland	, Member	**12/01/2010** Date Approved
Jon D. Rainier	, Member	**12/01/2010** Date Approved
Thomas G. Richmond	, Member	**12/01/2010** Date Approved
Ilya Zharov	, Member	**12/01/2010** Date Approved

and by **Henry S. White**, Chair of the Department of **Chemistry**

and by Charles A. Wight, Dean of The Graduate School.

ABSTRACT

Bryostatin 1 is a complex natural product isolated from the marine bryozon *Bugula neritina* by Pettit and coworkers whose structure was reported in 1982. This highly oxygenated macrolide has complex and unprecedented architectural features. In addition, bryostatin 1 has exhibited a remarkable biological profile including anticancer activity, synergetic effect with other anticancer agents, reversal of multidrug resistance, stimulation of the immune system, improvement of learning and memory, neuroprotection after stroke, reduction of amyloid plaque formation, and activity against HIV. The anticancer activity of bryostatin 1 has resulted in some 80 phase I and II clinical trials and a clinical trial against Alzheimer's disease is underway.

Despite having potential as a therapeutic drug, the availability of bryosatin 1 from natural as well as other sources is extremely limited. Synthesis of simplified analogues of bryostatin 1 provides an alternative way to solve the supply problem. With an aim of practical synthesis of simplified bryostatin 1 analogues, our group started the study of structure activity relationship of bryostatin 1 using a newly discovered pyran annulation methodology.

The work presented in this dissertation focuses on the synthesis, computational study and biological evaluation of bryostatin 1 analogues modified in the A and B ring region. Specifically, the role of three substituents in the A and B ring region has been

investigated by synthesizing C_{30}-decarbomethoxy bryostatin 1, C_9-deoxy bryostatin 1 and a C_8-gemdimethyl analogue. This study suggested that these three groups do not by themselves serve as functional switches between the PMA versus bryostatin 1 like activity of bryostatin analogues. Moreover, analogues with more polar groups in the A, B ring region tend to behave like bryostatin 1 as opposed to PMA and vice versa.

In addition to bryostatin analogues, the first total synthesis of bryostatin 1 was accomplished in 30 steps for longest linear sequence from commercially available starting material. The synthesis involved a highly convergent union of fully functionalized A and C rings by pyran annulation. The route developed to bryostatin 1 is also applicable for the synthesis of bryostatin 7, bryostatin 9 and bryostatin 15 as well as numerous bryostatin analogues.

This Dissertation Is Dedicated

To My Family

TABLE OF CONTENTS

STANDARD LIST OF ABBREVIATIONS

$\Delta\nu$	geometrical mean of the distances between the two outer and the two inner signals in an AB spin system (spectral)
[α]	specific rotation [expressed without units; units, deg mL/(g dm), are understood]
AB	AB spin system (spectral)
AIDS	acquired immunodeficiency syndrome
Ac	acetyl
appt	apparent (spectral)
ATP	adenosine triphosphate
9-BBN	9-borabicyclo[3.3.1]nonane
BINOL	(1,1'-binapthalene)-2,2' diol
BITIP	catalyst made by combining (1, 1'-binapthalene)-2, 2' diol and Ti(O*i*-Pr)$_4$
Bn	benzyl
BOC, Boc	*tert*-butoxycarbonyl
BOM	benzyloxy methyl
BPS	tertiary butyl diphenyl silyl
br	broad (spectral)
cLogP	computational log of partition coefficient

n-Bu	butyl
t-Bu	*tert*-butyl
Bz	benzoyl
°C	degrees Celsius
CAA	catalytic asymmetric allylation
calcd	calculated
CI	chemical ionization
CSA	10-camphorsulfonic acid
d	doublet (spectral)
DAG	diacyl glycerol
DCC	1,3-dicyclohexylcarbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
dr	diastereomeric ratio
DET	diethyl tartrate
DHP	dihydropyran
DIBAL	diisobutylaluminun hydride
DIPEA	diisopropylethylamine
DIPA	diisopropylamine
DMAP	4-dimethylaminopyridine
DMP	2,2-dimethoxy propane
DMF	dimethylformamide
DMP	dimethoxypropane
DMSO	dimethyl sulfoxide

ee	enantiomeric excess
Et	ethyl
EtOAc	ethyl acetate
EI	electron impact
g	gram(s)
GFP	green fluorescent protein
HMPA	hexamethylphosphoramide
HIV	human immunodeficiency syndrome
h	hour(s)
HPLC	high pressure liquid chromatography
HRMS	high-resolution mass spectrum
HRFAB	high-resolution fast atom bombardment
Hz	hertz
IC_{50}	50% inhibitory concentration
J	coupling constant (in NMR)
Ki	inhibitory dissociation constant
LAH	lithium aluminum hydride
LDA	lithium diisopropyl amide
LHMDS	lithium hexamethyldisilazide
LLS	longest linear sequence
LRMS	low-resolution mass spectrum
L-Selectride	lithium tri-*sec*-butylborohydride
M	moles per liter

*m*CPBA	*m*-chloroperoxybenzoic acid
Me	methyl
MHz	megahertz
min	minute(s)
mol	mole(s)
MOM	methoxymethyl
MEM	methoxy ethoxymethyl
Merle	american country music singer
MMPP	Magnesium monoperoxyphthalate
mp	melting point
MS	mass spectrometry, molecular sieves
Ms	methanesulfonyl
MTPA	α-methoxy-α-trifluoromethylphenylacetate
M/Z	mass to charge ratio (in mass spectrometry)
NaHMDS	sodium hexamethyldisilazide
NCS	N-chlorosuccinimide
nM	nano molar
NMO	*N*-methylmorpholine-*N*-oxide
NMR	nuclear magnetic resonance
PMA	phorbol 12-myristate 13-acetate
Ph	phenyl
PKC	protein kinase C
ppm	parts per million (in NMR)

PPTS	pyridinium *p*-toluenesulfonate
i-Pr	isopropyl
Py	pyridine
q	quartet (spectral)
SAR	structure activity relationship
R_f	retention factor (in chromatography)
rt	room temperature
s	singlet (spectral); second(s)
t	triplet (spectral)
TBAF	tetrabutylammonium fluoride
TBHP	tert-butyl hydroperoxide
TBS	*tert*-butyldimethylsilyl
TES	trimethylsilyl
TFA	trifluoroacetic acid
THP	tetrahydropyranyl
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl, tetramethylsilane
TPAP	tetrapropylammonium perruthenate
Ts	*p*-toluenesulfonyl
TsOH	*p*-toluenesulfonic acid

ACKNOWLEDGEMENTS

The work described in this dissertation would have been impossible without the contribution of several individuals. First, I would to like to express my sincere gratitude to my advisor Professor Gary Keck for allowing me to conduct research in his lab. Boss Keck's continuous guidance, support and encouragement over these years had tremendous impact on the completion of my graduate studies. I feel privileged to work with him and will always be proud of him as my scientific advisor.

I would like to thank Professor Jon Rainier, Professor Tom Richmond, Professor Illya Zharov and Professor Chris Ireland for guiding me during my graduate career.

I would like to thank Dr. Dennie Welch for helping me during my first year in the Keck lab. I am grateful to my colleagues Jeff Stephens, Arnab Rudra and Tom Cummins for their contribution in bryostatin program. Thanks are due to Matt Kraft, Wei Li and Xiguang Zaho for discussing the problems and helping each other out. I am appreciative of Mark Petersen for proofreading my thesis.

I would like take this opportunity to thank my family members for their love and patience. I am grateful to Pragya Adhikari for being such a wonderful wife. Last but not least, I am thankful to our little son Prayag for bringing a lot of happiness in our lives.

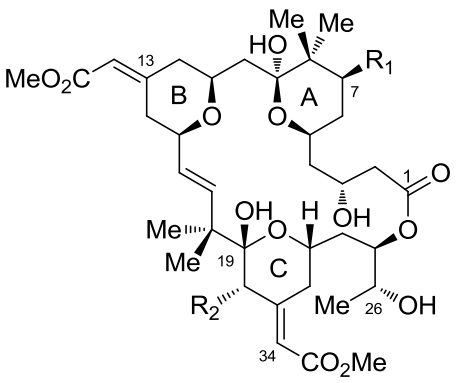
CHAPTER 1

THE SYNTHESIS AND BIOLOGICAL EVALUATION OF BRYOSTATIN ANALOGUES

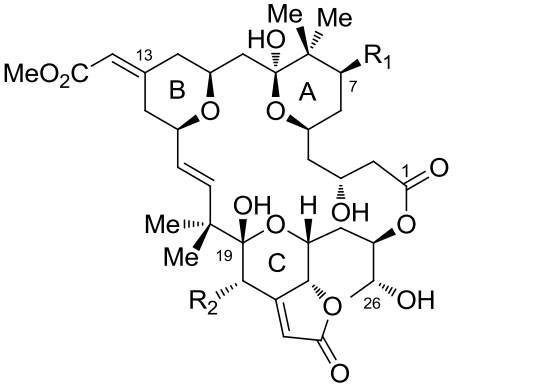
Introduction

In 1968, Pettit and coworkers detected that the extracts of marine bryozoans *Bugula neritina* had anticancer properties towards murine P388 lymphocytic leukemia cells.[1] After a large scale collection of the organism from the Gulf of Mexico and a bioassay guided fractionation of the animal extract, the active agent was identified as bryostatin 1, whose chemical structure was determined in 1982.[2]

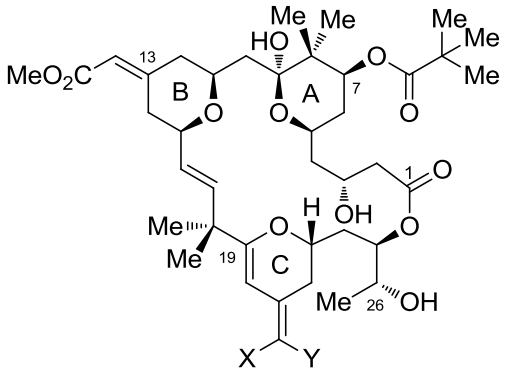
The bryozoan *Bugula neritina* is a filter feeder animal which forms mosslike colonies and is known to attach to ship hulls. Although bryostatin 1 was initially thought to be derived from *Bugula neritina*, it has been recently proposed that the symbiotic bacterium *Endobugula sertula* is the true source of the bryostatins.[3] The study revealed that bryostatins were highly concentrated on the larvae and are believed to act as an antifeedant providing chemical defense for the larvae of *Bugula neritina* against predation. On the other hand, the larvae of the *Bugula neritiana* provide a suitable medium for the growth of the bacteria.[4] Since the original isolation of bryostatin 1, nineteen other structurally related members have been isolated and characterized (Figure **1.1**).[5] The bryostatins are highly complex natural products characterized by the presence



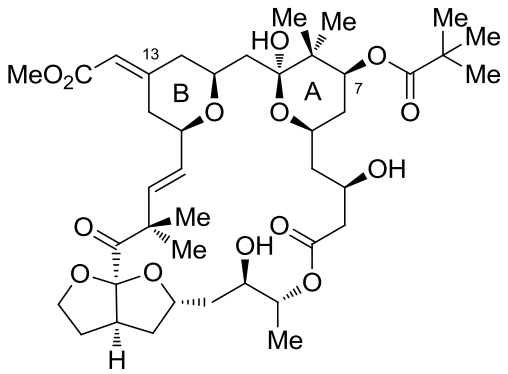
Bryostatin	R_1	R_2
1	OAc	$OCO(CH=CH)_2Pr$
2	OH	$OCO(CH=CH)_2Pr$
4	OCO*t*Bu	OCOPr
5	OCO*t*Bu	OAc
6	OCOPr	OAc
7	OAc	OAc
8	OCOPr	OCOPr
9	OAc	OCOPr
10	OCO*t*Bu	H
11	OAc	H
12	OCOPr	$OCO(CH=CH)_2Pr$
13	$OCO(CH)_4Pr$	H
14	OCO*t*Bu	OH
15	OAc	$OCO(CH=CH)_2CH(OH)Et$
18	OCO*t*Bu	H, *Z* C_{21}-C_{34} enoate



bryostatin	R_1	R_2
3	OAc	$OCO(CH=CH)_2Pr$
19	OCO*t*Bu	OCOPr
20	OCO*t*Bu	H



bryostatin	X	Y
16	H	CO_2Me
17	CO_2Me	H



neristatin 1

Figure 1.1. Bryostatin Family of Natural Products

of a twenty-membered macrolactone with eleven stereocenters and three embedded pyran rings, also known as the A-, B- and C-rings. The A- and C-rings of most bryostatins contain a hemiketal moiety whereas the B- and C-rings have an exocyclic α,β-unsaturated methyl esters. While all bryostatins have a gem-dimethyl group at C_8 and C_{18} positions, most of the bryostatins differ from each other at the C_7 and C_{20} positions on the A- and C-rings, respectively. Except bryostatin 2, which has a free hydroxyl group at the C_7 position, all the bryostatins have esters at the C_7 position. Similarly, most of the bryostatins also have an ester at the C_{20} position except bryostatins 10, 11, 18 and 20, which have no substituents at C_7 position. Some members of the family such as bryostatin 3, 19 and 20 have a more functionalized C-ring characterized by the presence of a five membered lactone fused with the pyran ring. Bryostatins 16 and 17, on the other hand, have less functionalized C-rings in which C_{19} and C_{20} functionality has been replaced with a glycal moiety. An additional variation is that the exocyclic methyl enoate in C-ring of bryostatins 17 and 18 have *E* geometry whereas all other bryostatins have *Z* geometry. Structurally different from the bryostatins, neristatin is another member of the family, which has a rearranged/oxidixed C-ring.[5] Among all bryostatins, bryostatin 1 is the most abundant in nature and the most studied member of the family.

The Biological Activity of Bryostatins

Since the first detection of its ability to inhibit the growth of the murine P388 lymphocytic leukemia cells, a number of studies have been carried out using bryostatin 1 as an anticancer agent. It has been found from these studies that bryostatin 1 is a highly potent anticancer agent that inhibits the growth of various human cancer cell lines at

remarkably low concentrations. As a result, bryostatin is in roughly 80 phase I and II human clinical trials as an anticancer chemotherapeutic agent. Data from the clinical trials have verified that bryostatin 1 is extremely potent anticancer drug requiring only about 1 mg of the drug over a course of 8 weeks treatment. By comparison, a similar 8-week treatment would require about 2.6 grams of Taxol. Despite being highly active, bryostatin 1 is quite well tolerated by humans, the only side effect being myalgia muscle pain or due to dose limiting toxicity. In addition to being used as a single chemotheraupetic agent, bryostatin 1 has exhibited impressive synergetic effects when used in combination with other well established oncolytic agents such as paclitaxel, vincristine, cisplatin and gemcitibine.[6] In contrast to most anticancer drugs, which suppress the immune system, bryostatin 1 has been shown to stimulate the immune system.[7] More recently, Chauhan and coworkers have shown that bryostatin 1 inhibits latent HIV infection in the lymphocytes indicating that bryostatin 1 has potential application in the context of acquired immune deficiency syndrome (AIDS).[8] Stimulation of the immune system by bryostatin 1 is believed to occur by promotion of T-cell proliferation.

Besides anticancer and immune-stimulant activity, another important biological activity of bryostatin 1 is its ability to affect neurological activity. Recent studies by Alkon and co-workers have shown that bryostatin 1 improves the learning and memory in animal models such as snails, rats and rabbits.[9] In a separate study, bryostatin 1 has been shown to reduce the formation of amyloid plaques, a key indicator of Alzheimer's disease, in transgenic mice.[10] Both of these studies indicate that bryostatin 1 has potential for the treatment of Alzheimer's disease and a human clinical trial is in progress.[11] In addition, bryosatin 1 has recently been found to possesses neurorepair activity. In

particular, bryostatin was able to reverse neural damage and restore spatial memory and learning in mice with ischemia/hypoxia induced stroke. [12]

The mode of action by which bryostatin 1 elicits these interesting biological activities is still under investigation; however, parts of these effects are believed to arise due binding of the bryostatin 1 to protein kinase C (PKC) and activating the enzyme. Bryostatin 1 binds with PKC with nanomolar affinity (K_i = 1.35 nM).[13] Protein kinase C is a family of kinases, that regulate various inter- and intracellular signal transduction processes through the phosphorylation of serine and threonine residues of substrate proteins. Physiologically, PKC is known to have an important role in various cellular processes such as proliferation, differentiation, motility, inflammation, and apoptosis. Irregularities in PKC signaling are linked to diseases such as cancer, diabetes, autoimmune disease, neurological disorders and cardiovascular diseases.[14]

PKC is comprised of a group of 10 isozymes that are divided into three categories based on the cofactors required for their activation (Figure **1.2**).[15] The conventional isozymes (α, βI, βII, γ) are activated by phosphatidyliserine (PS), diacylglycerol (DAG) and Ca^{+}, whereas the novel isozymes (δ, ε, η/L, θ) are activated only by PS and DAG. On the other hand, atypical isozymes (ζ, ι/λ) are activated only by PS. Only the conventional PKCs (cPKCs) and novel PKCs (nPKCs) are capable of binding with the ligands such as bryostatin, DAG, and phorbol esters. All the PKC isozymes contain an N-terminal regulatory domain and C-terminal catalytic domain connected by a proteolytic region. The catalytic domain, which is highly conserved among various isozymes, is responsible for the phosphorylation of the substrate protein. The regulatory domain has two parts, an

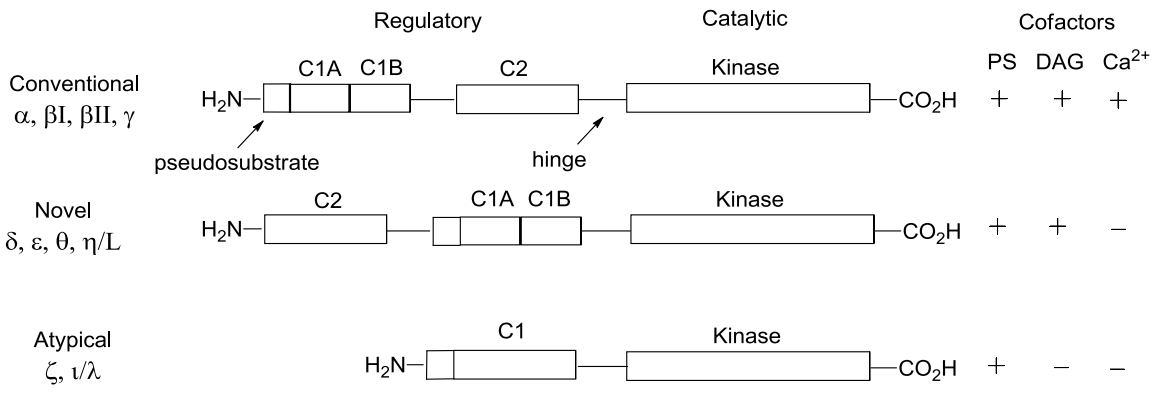


Figure 1.2. Schematic Representation of PKC Family

autoinhibitory pseudosubstrate region and a membrane targeting C region. While the pseudosubstrate region is common to all PKCs, the membrane targeting C region varies in each class of PKCs. The conventional PKCs have a cystine rich C1 (C1A and C1B) domain which binds to DAG and a calcium-binding C2 region. It is important to note that the C1 domain is present in other proteins such as RasGRP, chimaerins and Munc 13 and some of the biological activities of bryostatins are thought to be mediated by these proteins as well.[16] In the case the of nPKCs, not only are the C domains switched, but the C2 domain is inactive. In contrast to both cPKCs and nPKCs, the aPKCs do not have any C2 domain and the C1 domain is inactive.

The mechanism of PKC activation consists of two major events: (i) conversion of the newly synthesized PKC into its mature catalytically competent form by phosphorylation of the catalytic kinase domain and (ii) binding of a ligand (DAG, phorbol esters or bryostatin) to the regulatory domain (Figure **1.3**).[17] The newly synthesized PKC is freely floating in the cytosol and associates with the cytoskeleton.

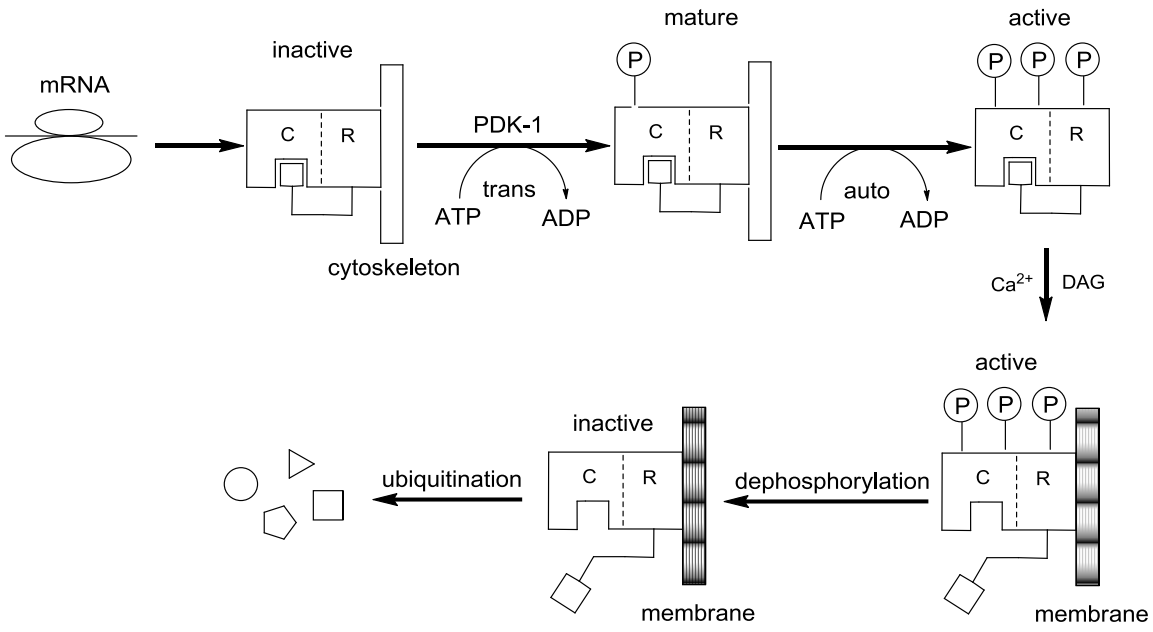


Figure 1.3. Schematic Representation of PKC Downregulation

Transphosphorylation by the phosphatidylinositol-dependent protein kinase 1(PDK-1) converts the PKC into its mature form. The mature PKC then undergoes two phosphorylation events. First autophosphorylation of the serine/threonine residues on the kinase domain is responsible for turning the PKC into its catalytically competent conformation whereas the second autophosphorylation releases the protein in the cytosol. The fully phosphorylated free floating PKC is inactive because the pseudosubstrate in the regulatory domain occupies the substrate binding site in the catalytic domain. On the other hand, certain stimuli cause the hydrolysis of the phosphatidylinositol 4,5 biphosphate into inositol triphosphate and DAG causing the release of Ca^{2+} from an intracellular organelle. Binding of the Ca^{2+} to the C2 region of PKC increase its affinity for negatively charged lipids and the PKCs translocate to the cell membrane. Once

associated with the cell membrane, the C1 domain of PKC binds with DAG which increases the hydrophobic surface and ensures a tight membrane association. The strong lipid association of PKC results in a conformational change releasing the pseudosubstrate region from the catalytic domain and thus fully activating the enzyme. In addition to endogenous ligands, PKC is also able to bind with external ligands like phorbol ester and bryostatins. Once fully activated, the enzyme begins its catalytic activity by binding and phosphorylating specific protein substrates rendering its biological activity. Ultimately, PKC is dephosporylated by membrane bound alkaline phosphatases, which convert it into catalytically inactive form. The inactive PKC is then degraded via ubiquitination resulting in down regulation.

In addition to diacylglycerols, which are endogenous activators of PKC, chemically very different structures such as phorbol esters (phorbol 12-myristate 13-acetate PMA, K*i* = 0.55 nM) and bryostatins are high affinity competitive ligands for PKC.[18] Unlike DAGs, which are relatively unstable under physiological conditions and are degraded quickly, bryosatin and PMA are rather robust to degradation. This causes the long term activation of PKC ultimately leading to its downregulation.[19] Although both phorbol esters and bryostatins bind to PKC and cause its activation, the resulting biological effect of these ligands is different and unique.[20] Bryostatin 1 induces only a few of the responses exhibited by phorbol esters. Moreover, bryostatin blocks those responses which it does not induce itself or those induced by the phorbol ester. One of the most important examples of such antagonism is that phorbol esters are tumor promoters whereas bryostatin inhibits the tumor promotion induced by phorbol esters.[21] The

mechanism by which phorbol esters and bryostatins exhibit such antagonism is not yet understood.

Development of Analogues of Bryostatin 1

Because of their diverse biological activities, bryostatins present an excellent lead for therapeutic development. However these natural products are extremely scarce in nature and not readily available. The largest isolation of the bryostatins from 1300 kg of *Bugula neritina* provided only 18 grams of bryostatin 1 with a yield of 0.0013%.[22] This material is being used for all the biological and clinical studies to date. The isolation of bryostatins from such a nonrenewable source is not viable for long term purposes and has serious ecological consequences. Attempts to aquaculture the organism *Bubula neritina* in an artificial environment by CalBiomarine Technologies were not cost effective due to low production of bryostatins and the company ultimately stopped its operation.[23] Since the discovery of symbiotic bacteria *Endobugula sertula* as the true source of the bryostatins, effort has been devoted to culture the bacteria. Unfortunately, the symbiotic bacterium has proven difficult to grow in the absence of its host.[24] Another potential method of producing bryostatins is via a biosynthetic route which involves cloning of the genetic code for bryostatin from the source organism and transplanting and overexpressing it in a suitable host. Studies in this area are underway and have so far been able to identifiy and isolate the putative bryostatin gene cluster form *Endobugula sertula*.[25] Even if successful, this would afford a rather "bare bones" bryopyran dubbed "bryostatin zero", and not (even) one of the more highly oxidized natural bryostatins. On the other hand, the impressive biological activity coupled with challenging structure and

low natural abundance of bryostatins have attracted a number of groups towards their total synthesis. This has resulted in the total synthesis of bryostatins 7, 2 and 3 by the Masamune, Evans and Yamamura groups respectively.[26,27,28] However due to the large size and complex nature of these natural products, these total syntheses required more than 70 chemical steps and were not able to provide clinically relevant quantities of material. Very recently, a relatively concise synthesis of the simpler and less well studied bryostatin 16 has been reported by Trost and coworkers but provided less than a milligram of the final product.[29]

The restricted availability of bryostatins both naturally and synthetically has affected its further development as therapeutic agent. Bryostatin 1 is not produced by nature as an optimized therapeutic drug, but rather as a means of chemical defense. However, bryostatins can serve as a lead for the development of more clinically useful drugs. Optimization of such a lead structure involves careful modifications of the parent compound generating simpler analogues which retain or exceed the biological properties of the parent compound. Moreover, such simplification would allow the practical preparation of the analogues in a more efficient manner. Prior to any modification of the lead compound, it is necessary to identify the critical structural elements responsible for the desired biological activity.

The fact that DAGs, phorbol esters and bryostatins bind to the same site of the PKC led to the hypothesis that they may share a common pharmacophoric region (Figure **1.4**). Studies based on this hypothesis were carried out under a collaborative effort among the Wender, Pettit and Blumberg groups using computational and chemical studies of these PKC activators.[30] At this point, the SAR studies of PMA had shown that retention

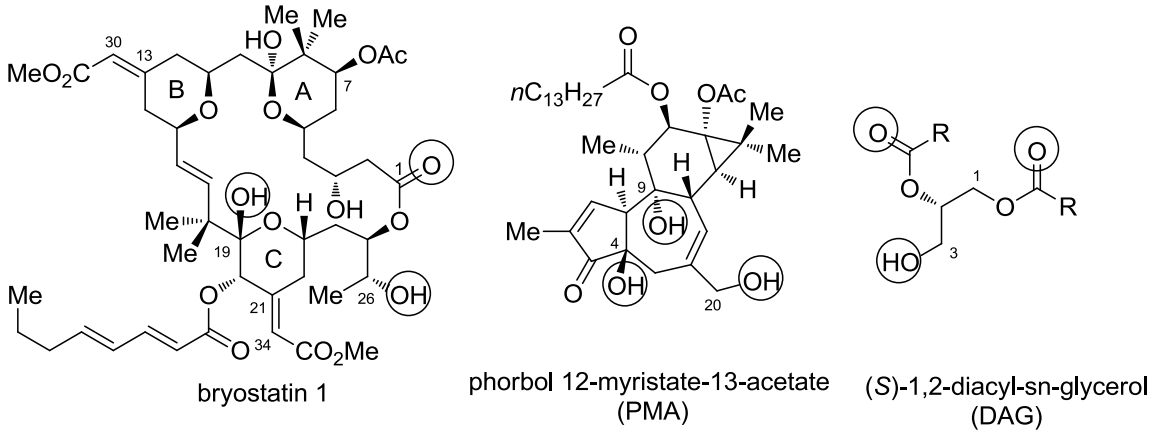
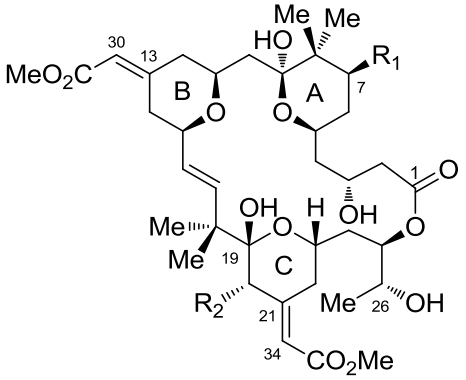


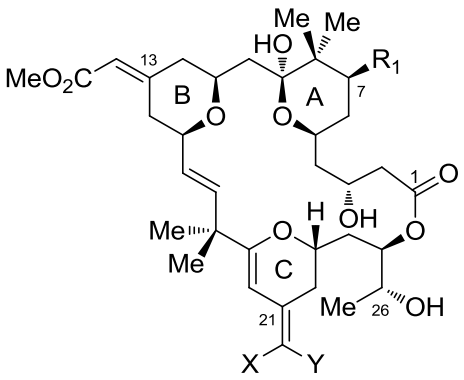
Figure 1.4. Pharmacophores of Some PKC Activators

of tumor promoting activity required the presence of a long chain hydrocarbon at C_{12}/C_{13} alcohol and free hydroxyl groups at C_4 and C_9. A comparison of the spatial coordinates of the lowest energy conformations of (*S*)-1,2-diacyl-*sn*-glycerol to the X-ray crystal structure of PMA showed that the two carbonyl and alcohol oxygen of the DAG correlated with that of C_4, C_9 and C_{20} oxygen atoms of PMA. A similar comparison between the crystal structure of PMA and of bryostatin 1 revealed a close relationship between the C_4, C_9 and C_{20} oxygen atoms and the C_1 carbonyl, C_{19} and C_{26} alcohol of bryostatin 1, respectively. Mapping of such interatomic distance thus suggested that the requirement of C_1-C_{19}-C_{26} heterotriad was necessary for biological activity.

Next, a comparison of the PKC binding affinity of the bryostatins 1-11 revealed that that substitution at C_7 and C_{20} only moderately changed the binding affinity (Figure **1.5**). On the other hand, bryostatin 16 and 17, which lack the C_{19}-C_{20} oxidation, had binding affinity 80 to 130 times less than that of bryostatin 1 indicating that this region is necessary for binding affinity. Additionally, the semisynthetic analogues derived from



bryostatins



X = H, Y = CO_2Me, bryostatin 16
X = CO_2Me, Y = H, bryostatin 17

Bryostatins	R_1	R_2	K*i* for PKC (nM)
1	OAc	$OCO(CH=CH)_2Pr$	1.4
2	OH	$OCO(CH=CH)_2Pr$	5.9
3			2.8
4	OCO*t*Bu	OCOPr	1.3
5	OCO*t*Bu	OAc	1.0
6	OCOPr	OAc	1.2
7	OAc	OAc	0.8
8	OCOPr	OCOPr	1.7
9	OAc	OCOPr	1.3
10	OCO*t*Bu	H	1.6
11	OAc	H	4.8
16			118
17			188
18	OCO*t*Bu	H, *Z* C_{21}-C_{34} enoate	4.8
2[a]	OH	$OCO(CH_2CH_2)_4Pr$, C_{13}-C_{30} dihydro	9.6
2[b]	OH	$OCO(CH_2CH_2)_4Pr$, C_{13}-C_{30} C_{21}-C_{34} tetrahydro	473
4[a]	OCO*t*Bu	OCOPr, C_{13}-C_{30} epoxide	0.54
4[b]	OCO*t*Bu	OCOPr, C_{26} acetate	>>100
1[a]	OAc	$OCO(CH=CH)_2Pr$, C_{26} epi	32.6

Figure 1.5. Binding Affinity of Bryostatins and Semisynthetic Analogues

the hydrogenation or epoxidation of the C_{13}-C_{30} olefin of the bryostatins had affinity compared to that of natural products whereas hydrogenation of the both C_{13}-C_{30} and C_{21}-C_{34} olefins significantly reduced the binding affinity. Removal of the unsaturation from the side chain did not have much effect in the binding affinity. Finally inversion or acetylation of the C_{26} stereochemistry significantly diminished the binding affinity. These studies thus supported the hypothesis from the computational studies that C_{19}-C_{26} region of bryosatin 1 is important for the binding with PKCs.

Results from the computational studies taken together with the SAR obtained from the binding affinity of the natural product as well as the semisynthetic analogues indicated that the southern C-ring region of the bryostatin 1 binds with the PKC. It was also hypothesized that the northern A-B-ring region as well as the hydrocarbon chain on the C-ring hold the pharmacophoric southern region in a rigid conformation providing a proper orientation for binding with PKC. The two regions were then coined by Wender as "spacer domain" and "recognition domain" for A-B-ring and C-ring regions, respectively.

Wender's Design of Bryosatin Analogues

Based on pharmacophore model mentioned above, Wender and coworkers have designed and synthesized analogue **1.1** in which all of the functional groups on the A and B-rings have been eliminated (Figure **1.6**).[31] Moreover, the B-ring pyran is replaced by a cyclic acetal for the ease of synthesis. The other difference between this analogue and bryostatin 1 is removal of unsaturation from the side chain on the C-ring. Their design of lead analogue **1.1** is shown in Figure **1.6** where the macrolactone was envisioned to form via an intermolecular esterification of the recognition domain alcohol **1.2** with the spacer

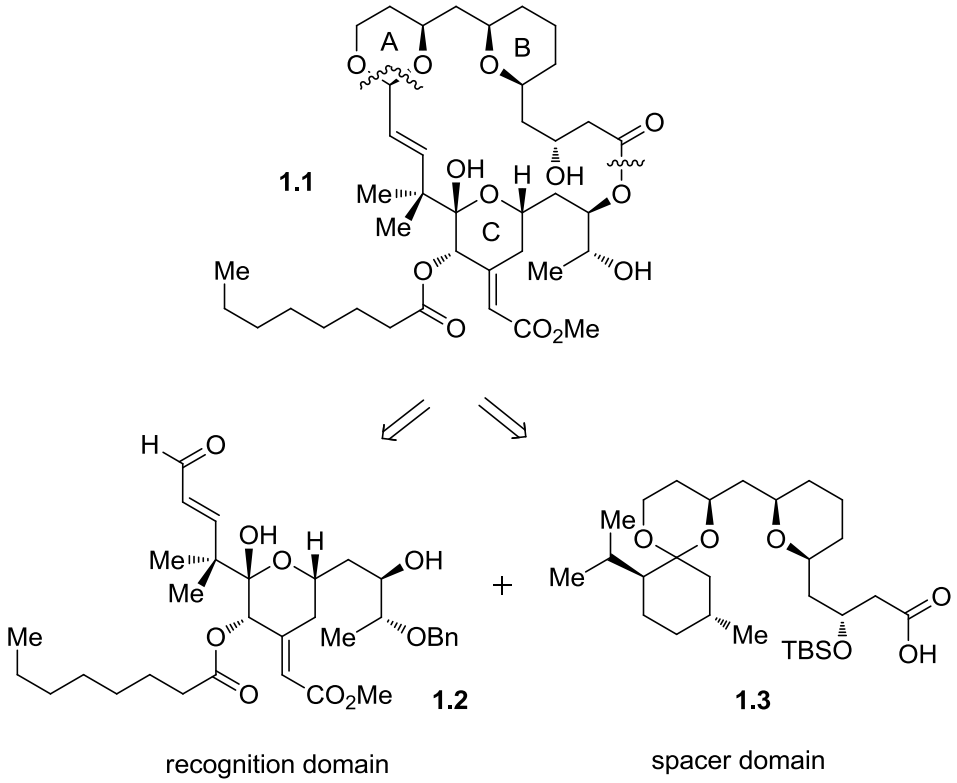


Figure 1.6. Wender's Retrosynthesis of Bryostatin Analogue **1.1**

domain carboxylic acid **1.1**. The esterification would be followed by an acid catalyzed transacetalization that would form B-ring and the macrolactone as well remove the protecting groups.

The first generation synthesis of the recognition domain **1.2** commenced with benzyl protection of commercially available (*R*) methyl lactate (Figure **1.7**). Half reduction of the methyl ester to the aldehyde followed by a 1,2 chelation controlled allylation installed the C25 stereocenter. The newly formed alcohol was protected as aPMB ether and the olefin was cleaved to an aldehyde using ozonolysis. In a separate set of reactions, ketone **1.8** was prepared in three steps from commercially available isopropyl methyl ketone **1.4**. An aldol reaction of the ketone **1.8** with acetaldehyde

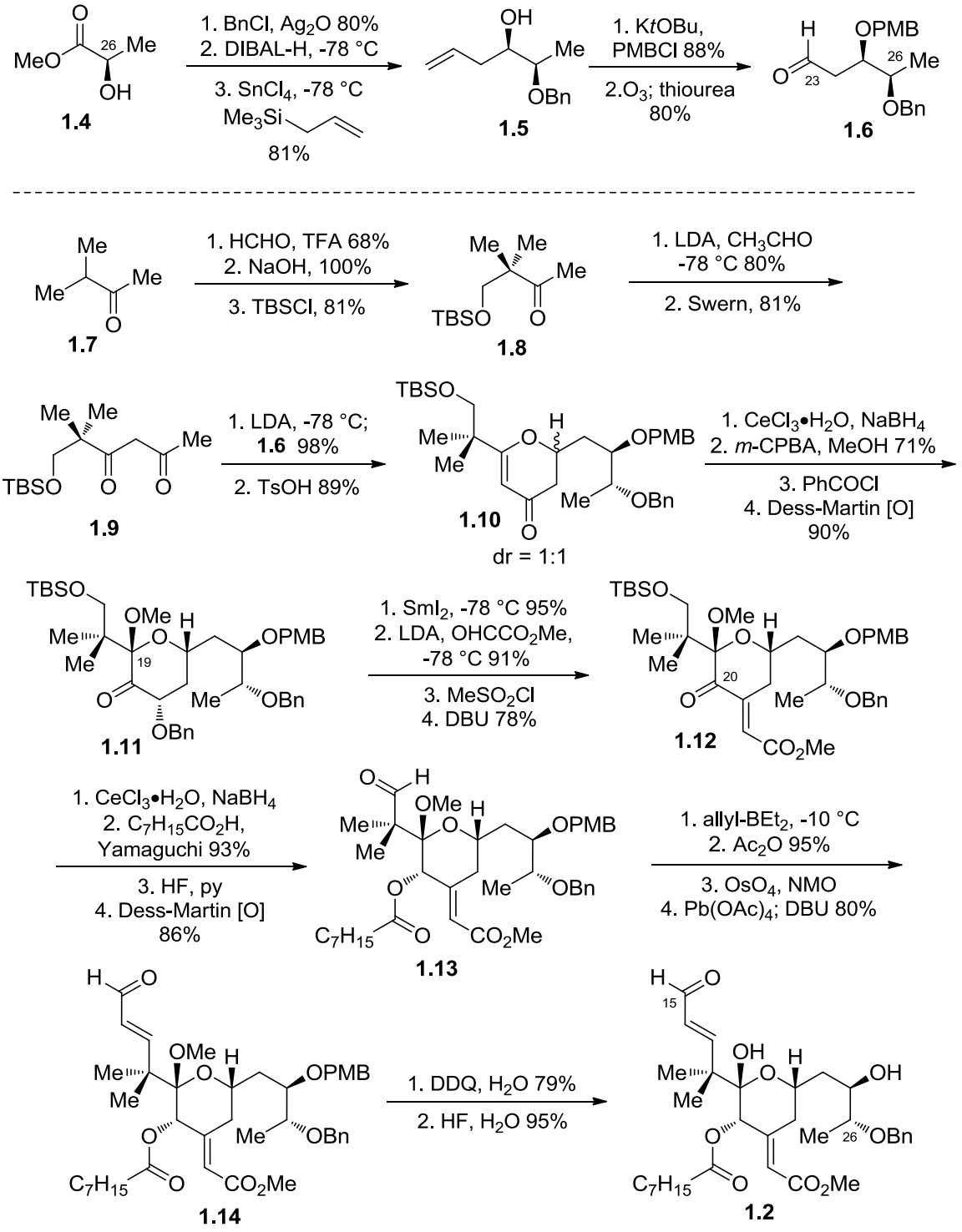


Figure 1.7. Wender's Synthesis of Recognition Domain **1.2**

provided the corresponding aldol adduct which was oxidized to a ketone **1.9**. The addition of the dienolate of diketone **1.9** to aldehyde **1.6** followed by dehydration provided a 1:1 mixture of pyranones **1.10,** which were separated using silica gel column chromatography. The desired β-isomer was subjected to Luche reduction. Epoxidation/methanolysis of the glycal followed by selective benzyl protection of the least sterically hindered alcohol and oxidation of the remaining C_{20} alcohol provided the ketone **1.11**. Deoxygenation of the benzyl ester provided the ketone which was subjected to an aldol reaction with methyl glyoxylate. The resulting aldol adduct was mesylated, and elimination of the mesylate using DBU provided the α,*β*-unsaturated methyl ester **1.12** as a single isomer. A Luche reduction of the C_{20} ketone followed by Yamaguchi esterification with octanioc acid installed the side chain on the C-ring. The removal of the TBS protecting group and subsequent oxidation of the alcohol provided aldehyde **1.13**. Addition of allyl-BEt_2 followed by acetylation of the resulting alcohol provided inconsequential mixture of acetates. Oxidative cleavage of the terminal olefin using OsO_4/$Pb(OAc)_4$ followed by DBU mediated elimination installed the α,*β*-unsaturated aldehyde. The PMB group was removed under oxidative conditions and hydrolysis of the methylketal completed the synthesis of recognition domain **1.2** in 25 steps (longest linear sequence).

In order to synthesize the spacer domain **1.3** (Figure **1.8**), the bisolefin **1.15** was converted into a triol by ozonolysis and in situ reduction presence of a Chiral catalyst providing the pyranone **1.17** as 2:1 mixture of diastereomers in favor of the desired isomer. Luche reduction of the ketone gave a *β*-alcohol which was converted into a vinyl ether, which upon heating underwent a [3,3] sigmatropic rearrangement providing the

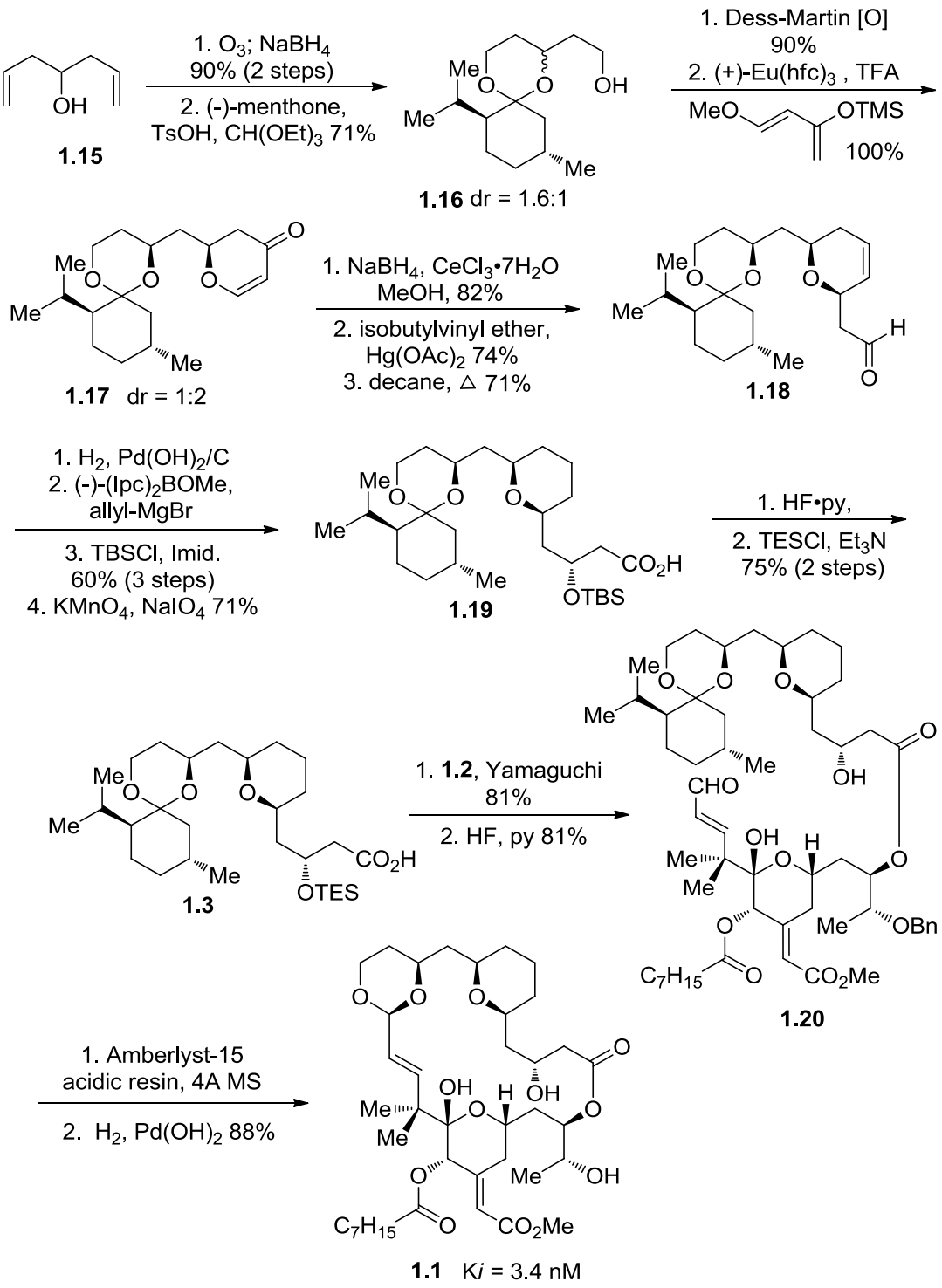


Figure 1.8. Wender's Completion of First Generation of Bryostatin Analogue

aldehyde **1.18**. Hydrogenolysis of the olefin, followed by Brown asymmetric allylation installed the C_3 stereocenter. The alcohol was protected as a TBS ether and oxidative cleavage of the olefin followed by oxidation of the resulting aldehyde to a carboxylic acid completed the synthesis of the spacer domain **1.3**.

Once both the spacer and the recognition domains were ready, the coupling of the carboxylic acid **1.3** with the alcohol **1.2** was accomplished using a Yamaguchi esterification. Removal of the TES group followed by treatment of the enal **1.20** with Amberlyst-15 acidic resin formed the B-ring acetal as single diastereomer. Removal of the benzyl protecting groups furnished the analogue **1.1** in 29 longest linear steps.

Biological evaluation of this analogue was carried out by measuring the binding affinity with the PKC mixtures isolated from rat brain. As suggested by the pharmacophoric model, the analogue **1.1** displayed high binding affinity (3.4 nM). In addition, the analogue **1.1** also showed 1.8-170 ng/mL growth inhibitory effect against several human cancer cell lines.

Wender's second generation analogue differed from the analogue **1.1** in just one position, i.e., the absence of C_{27} methyl group in the recognition domain.[32] This methyl group was deleted to ease the synthesis of the recognition domain. The second generation synthesis of the C-ring was accomplished in 18 steps from the diol **1.21** (Figure **1.9**).

Monoprotection of the diol, oxidation, Grignard addition and alcohol oxidation provided the keto-aldehyde **1.22**. The C_{23} stereocenter was installed using Keck asymmetric allylation providing the homoallylic alcohol in good yield and excellent enantioselectivity.[2] Dehydrative cyclization of the keto-alcohol followed by an epoxidation and *in situ* methanolysis provided the methoxy alcohol which was oxidized

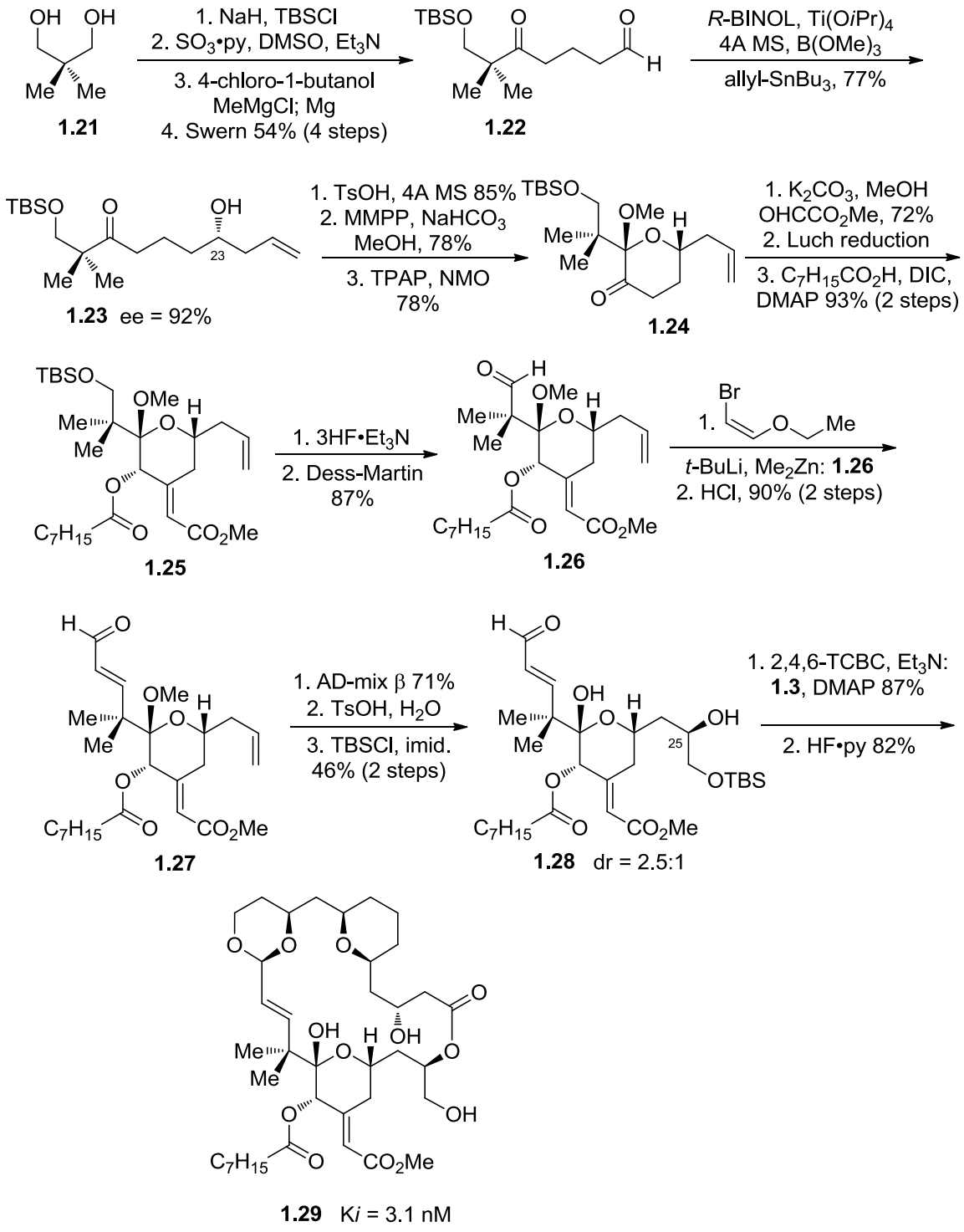


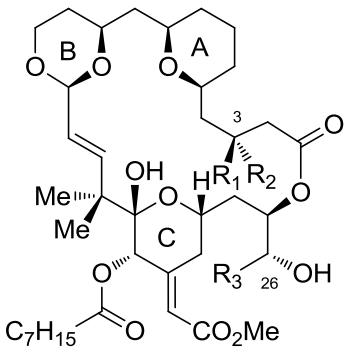
Figure 1.9. Wender's Synthesis of Second Generation of Bryostatin Analogue

to ketone **1.24**. The methyl enoate moiety was then installed using a aldol condensation with methyl glyoxylate. Reduction of the ketone under Luche condition followed by esterification with octanoic acid installed the saturated C_{20} side chain. A two carbon homologation of the aldehyde furnished the α,β-unsaturated aldehyde **1.37**. Sharpless asymmetric dihydroxylation of the terminal olefin provided a 2.5:1 mixture of diols favoring the desired isomer which were separated after the hydrolysis of the C_{19} methylketal and TBS protection of the primary alcohol. This completed the second generation synthesis of the "recognition domain" **1.28**.

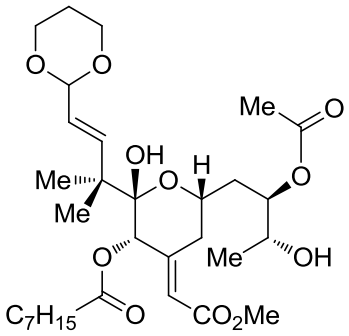
With this new recognition domain, the coupling of the carboxylic acid **1.3** with the alcohol **1.28** was accomplished using Yamaguchi esterification (Figure **1.9**). Treatment of the ester with HF in pyridine formed the B-ring acetal as well as removed the protecting groups furnishing the analogue **1.29** in 19 longest linear steps. Biological evaluation of analogue **1.29** was carried out similarly to analogue **1.1**. Although the binding affinity of this analogue was originally reported to be 0.25 nM, a more recent paper by Wender and coworkers report that the binding affinity is actually 3.1 nM.[33]

In addition to these analogues, Wender and coworkers have synthesized a number of other bryostatin analogues (Figure **1.10**).[34] Some representative analogues are shown in Figure **1.10** and for detailed chemical and biological descriptions, interested readers are referred to the review.[35]

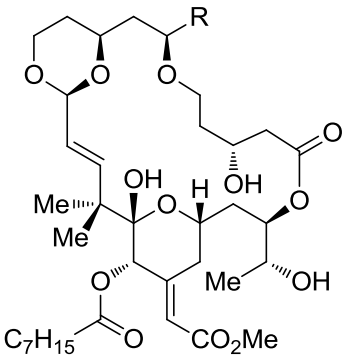
Several interesting observations were made from the biological evaluation of these analogues. Starting from the C_3 position, absence of the hydroxyl group or inversion of the stereochemistry at this position dramatically reduces the binding affinity towards PKC, presumably due to disruption of the intramolecular H-bonding. Analogues



	R_1	R_2	R_3	K*i* (nM)
1.30	R_1	R_2	R_3	K*i* (nM)
1.31	H	H	Me	297
1.32	OH	H	Me	385
1.33	H	OH	H	3.1

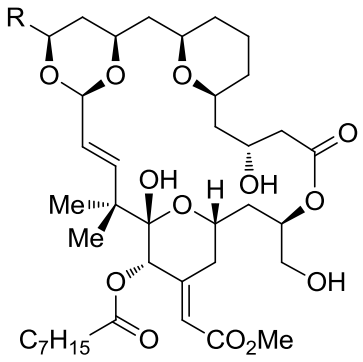


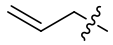
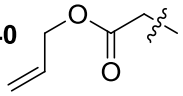
1.34 K*i* >10000 nM

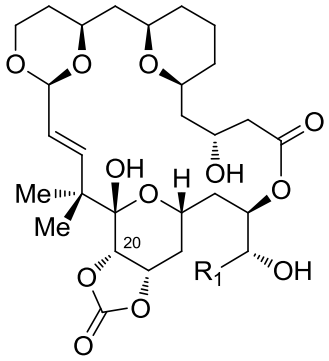


XX

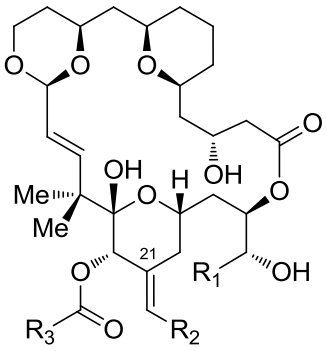
	R	K*i* (nM)
1.35	R	K*i* (nM)
1.36	H	47
1.37	*t*Bu	8.3
1.38	H	3.1



	R_1	K*i* (nM)
1.39		0.67
1.40		1.2



1.41 K*i* >10000 nM



	R_1	R_2	R_3	K*i* (nM)
1.42	H	Ph	C_7H_{15}	100
1.43	H	CO_2Bn	C_7H_{15}	120
1.44	Me	CO_2Me	Me	232
1.45	Me	CO_2Me	Ph	7

Figure 1.10. Representative Wender's Analogues

without the macrocyclic structure have severely low affinity, but the analogues with macrocyclic structure exhibited high binding affinity. Substitution of the B-ring acetal at the C_{13} position or its contraction to a five membered acetal does not affect the affinity. On the C-ring, removal of C_{20}-C_{21} unsaturation has a severe effect. Incorporation of bulky esters on the C_{21}-C_{34} segment significantly decreases the binding affinity.

Keck's Design of Bryostatin Analogues

The discovery of ever growing unique and interesting biological activities of bryostatins coupled with its limited natural abundance and challenging architecture had attracted our group towards the total synthesis of bryostatins and their analogues. The polyacetate-polypropionate nature of bryostatins provides an excellent opportunity for the application of the methodologies developed in our lab over the past years. The synthesis of simpler analogues of the bryostatin 1 not only enabled us to explore the SAR of these highly complex compounds, but also served as a model for the total synthesis of the natural product itself.

Our efforts directed towards the synthesis of bryostatin 1 has resulted in the development of a new reaction called "pyran annulation" which allows for the rapid asymmetric assembly of *cis*-2,6-dialkyl-4 methylene pyrans, a structural motif common to the B-ring of the bryostatins.[36] The reaction involves the construction of a β-hydroxyallyl silane **1.48** via CAA reaction of an aldehyde **1.46** with trimethylsilyl methylallylstannane **1.47** (Figure **1.11**). Treatment of such a silane **1.48** and aldehyde

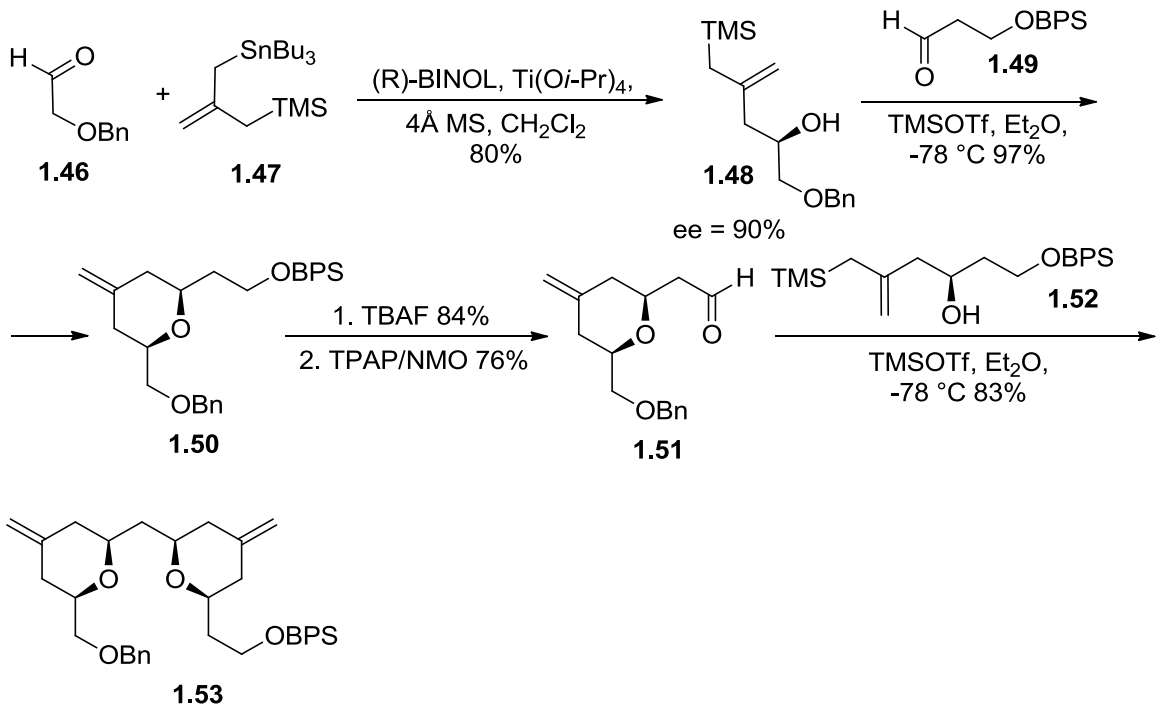


Figure 1.11. Pyran Annulation Methodology

1.49 with TMSOTf results the formation of the *2,6*-disubstituted 4-methylene pyran **1.50**. Figure **1.11** shows the application of this methodology in the construction of bispyran compound **1.53** as demonstrated previously by Dr. Jonathan Covel from our group.

If A- and B-rings of bryostatin 1 is simply spacer domain as suggested by Wender, replacement of the highly functionalized A-B-rings of the bryostatin 1 by bispyran **1.53** would result a bryopyran analogues with biological activity comparable to that of bryostatin 1. In addition, application of the pyran annulation methodology should allow the synthesis of simplified analogues in a rapid manner. With this concept, our group started the synthesis of bryostatin analogues in which the A- and B-rings would be simplified to the exo-methylene pyrans as in **1.53**.

This approach towards the synthesis of bryostatin analogues was initiated by Dr. Anh Truong (Figure **1.12**).[37] Using a sequential pyran annulation approach, Dr. Truong was able to synthesize bryopyran analogue **1.62**. Biological evaluation of the analogue **1.62** in terms of binding affinity was carried out by Dr. Peter M. Blumberg from NIH. The analogue **1.62** was found to have a inhibitory dissociation constant (K*i*) of 546 nM with PKC-α. Such higher dissociation constant indicated that analogue **1.62** had weaker

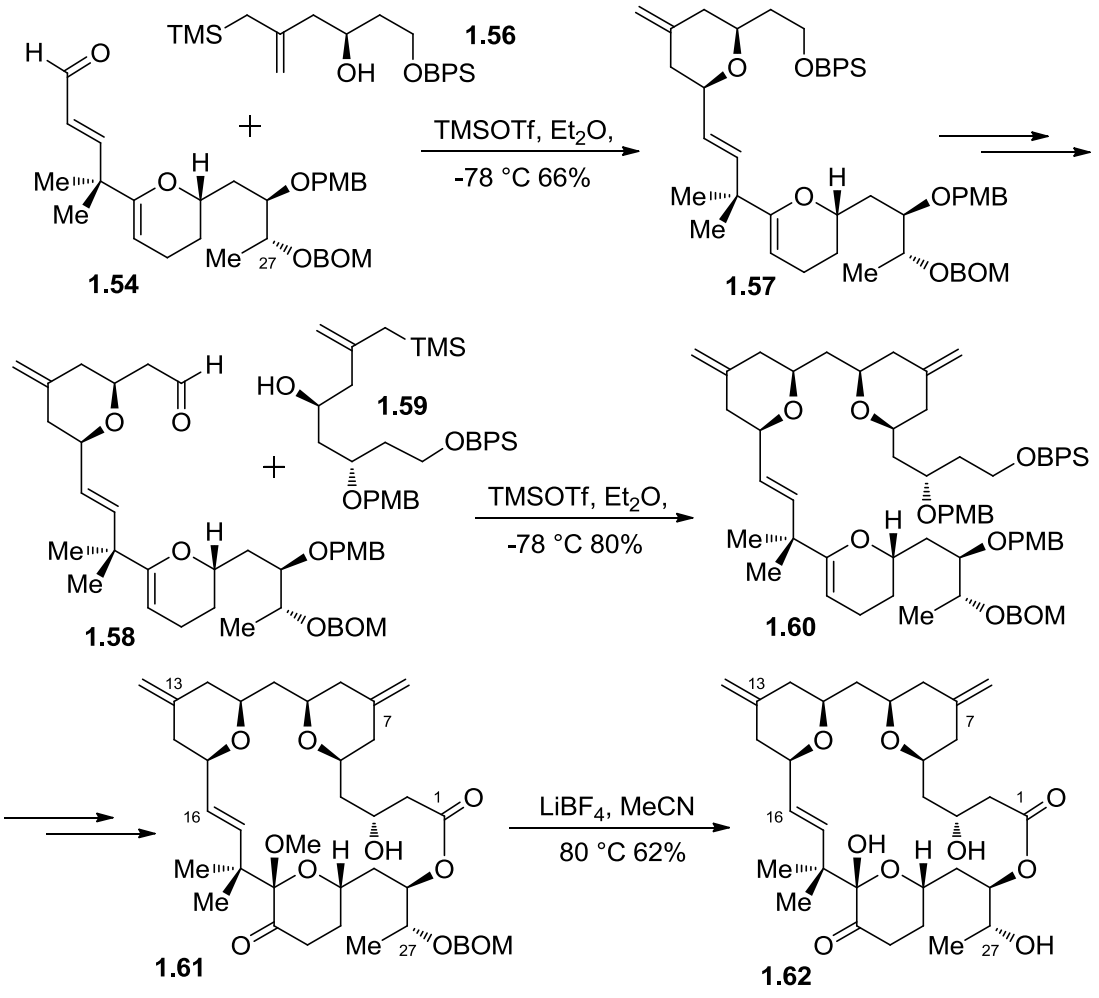


Figure 1.12. Dr. Anh Truong's Synthesis of Bryostatin Analogue 1.62

binding affinity towards PKC than does bryostatin 1 (K*i* = 1.35 nM). This study suggested that lack of functional groups in the C-ring of the analogue **1.62** might be responsible for such decrease in the binding affinity.

At this point, Dr. Carina Sanchez took the intermediate **1.61** (Figure **1.13**) and fully functionalized the C-ring. This involved the protection of the C_3 alcohol with TBS group followed by an aldol reaction of the C-ring ketone with methylglyoxylate.

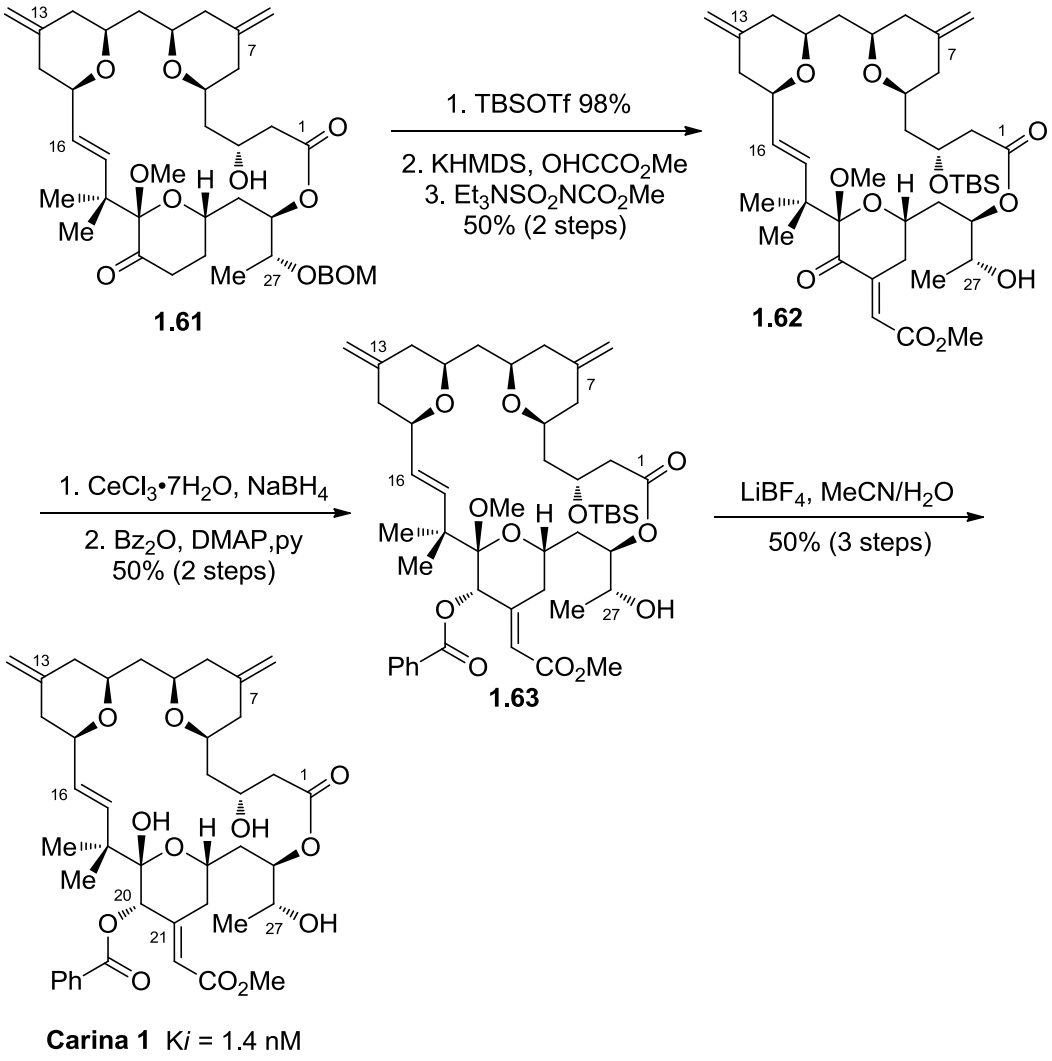


Figure 1.13. Dr. Carina Sanchez's Synthesis of Bryostatin Analogue Carina 1

Elimination of the aldol adduct gave α,β-unsturated keto ester and the ketone was reduced under Luche conditions. Esterification of the resulting alcohol with benzyoic anhydride followed by global deprotection completed the analogue "Carina-1" .[38] This analogue has similar substituent at C_{21} position and a different ster in C_{20} position compared to C-ring of bryostatin 1. The binding affinity of Carina-1 (K*i* = 1.35 nM) was found similar to that of bryostatin 1 indicating the necessity of the C_{20} and C_{21} functional groups. Although analogue Carina-1 has binding affinity comparable to that of bryostatin 1, it does not truly reflect its biological function. This is because bryostatins and PMA both bind competitively to the same C1 domain of the PKC with different end results. Bryostatin 1 is not a tumor promoter whereas PMA is. Thus, it was necessary to determine whether Carina-1 behaves like bryostatin or not. Biological function of the Carina-1 was determined using U937 human leukemia cell line. This cell line exhibits different behavior towards PMA and bryostatin 1. For example, PMA induces attachment and inhibits the proliferation of U937 cells in dose dependent manner whereas bryostatin has little effect.[39] Moreover, bryostatin blocks the effect of the PMA in dose dependent manner. When Carina-1 was tested using these assays (Figure **1.14**), it displayed activity similar to PMA indicating that it mimicked the biological activity of PMA despite being a close structural analogue of bryostatin 1.

The difference between bryostatin 1 and Carina-1 lies in five positions, three positions on the A-ring and one each on the B- and C-rings (Figure **1.15**). Since Carina-1 behaves like tumor promoting PMA, these five groups alone or in combination must be responsible for such a switch in biological function. Thus, we directed our study to determine the role of these groups through the synthesis and biological evaluation of

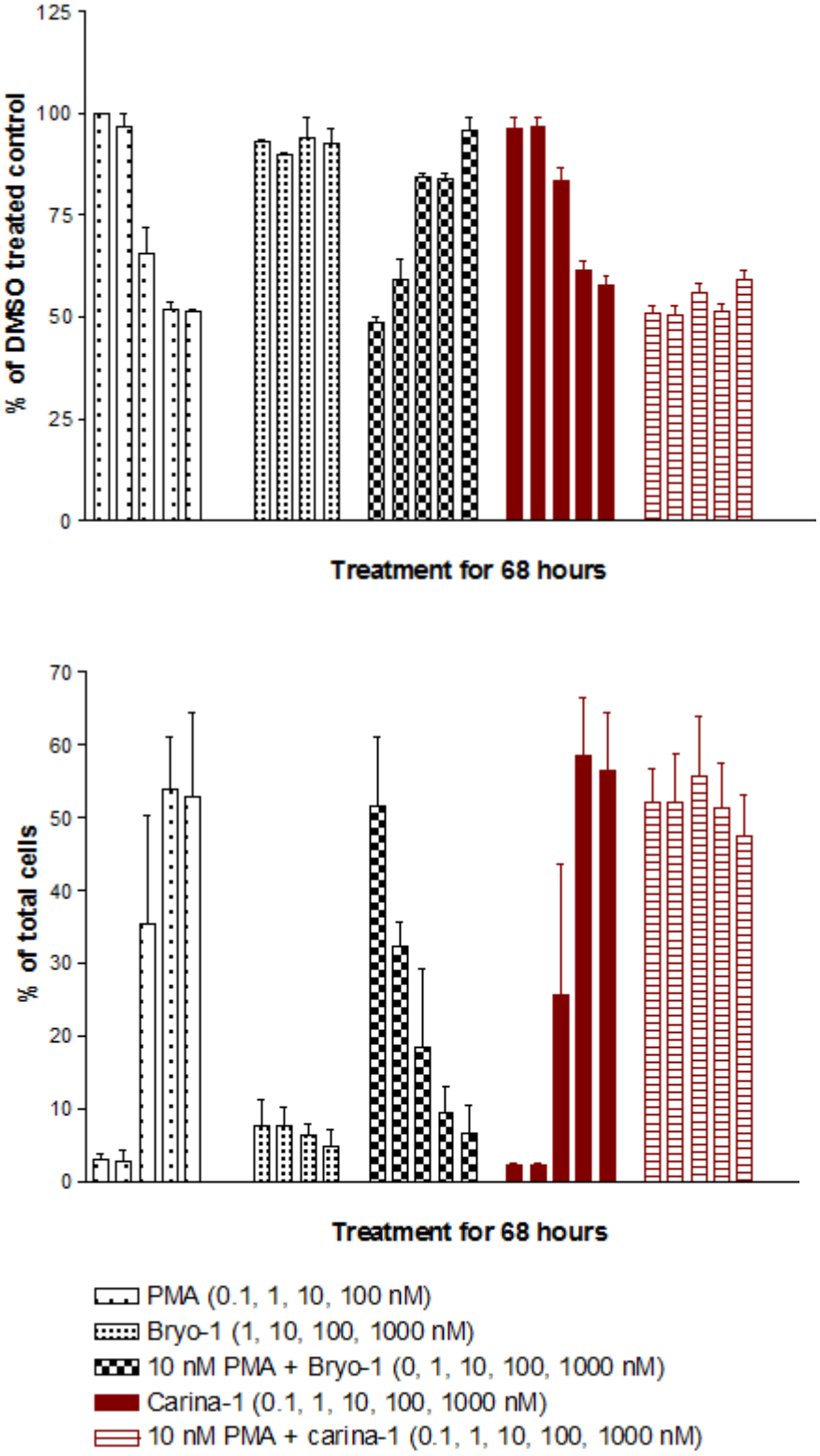


Figure 1.14: Proliferation (top) and Attachment (bottom) of U937 Cells by **Carina-1**

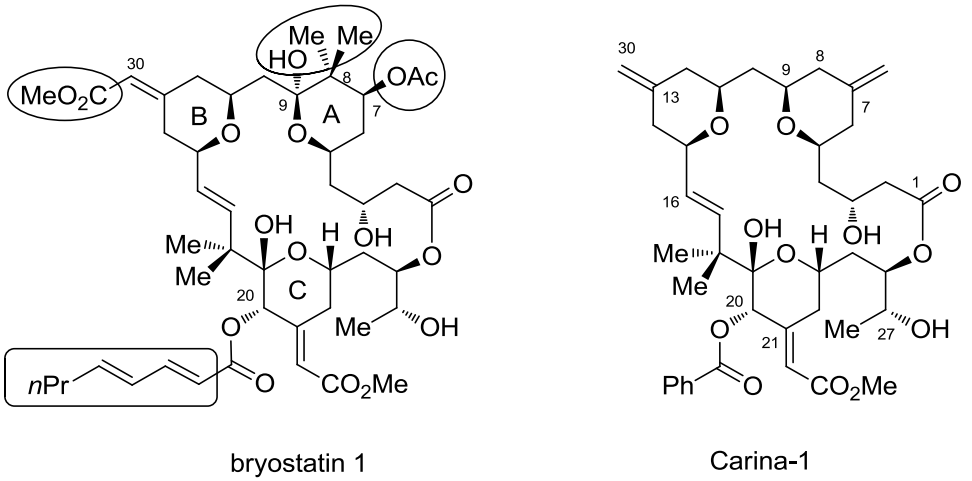


Figure 1.15. Difference Between Bryostatin 1 and Carina-1

analogues varying in these positions.

From the synthetic point of view, it seemed more reasonable to determine the role of C_{21} ester first since this would require only a minor modification on the existing route. Using a more convergent and improved pyran annulation approach, Matthew B. Kraft and Wei Li from our group synthesized three analogues with different side chains at the C_{20} position (Figure **1.16**).[40] All of these analogues were found to have binding affinity slightly better than bryostatin 1 indicating the tolerance of different ester side chains at C_{20} position. Further biological activity in terms of function was carried out using U937 proliferation and attachment assays as mentioned before (Figure **1.17**). To our surprise, Merle 23 also behaved similar to PMA but different from bryostatin 1 in these two assays. Since bryostatin 1 and Merle 23 differ only at four positions on the A and B region, this region must somehow be responsible for PMA like activity of Merle 23. If the A- and B- rings of the bryostatin 1 were simply spacer domain as suggested by

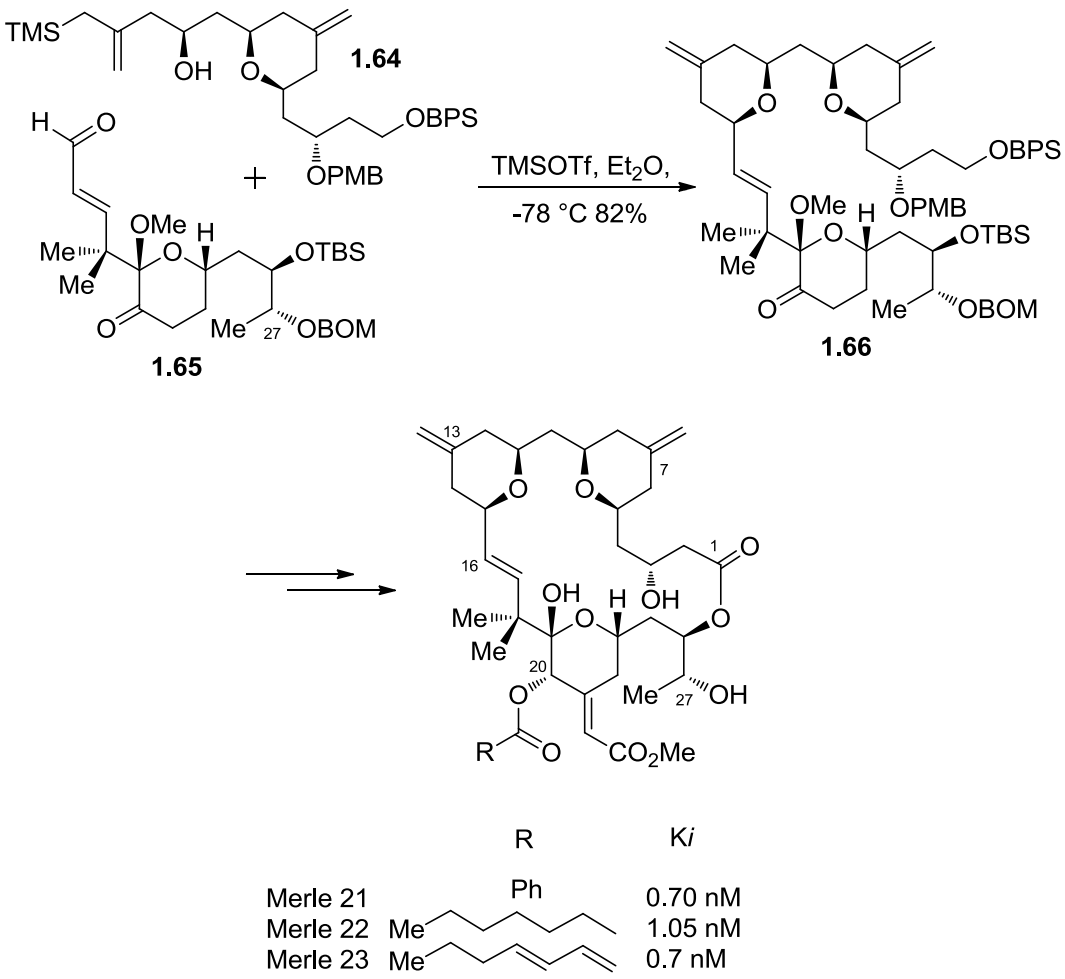


Figure 1.16: A More Convergent Approach to Bryostatin Analogues

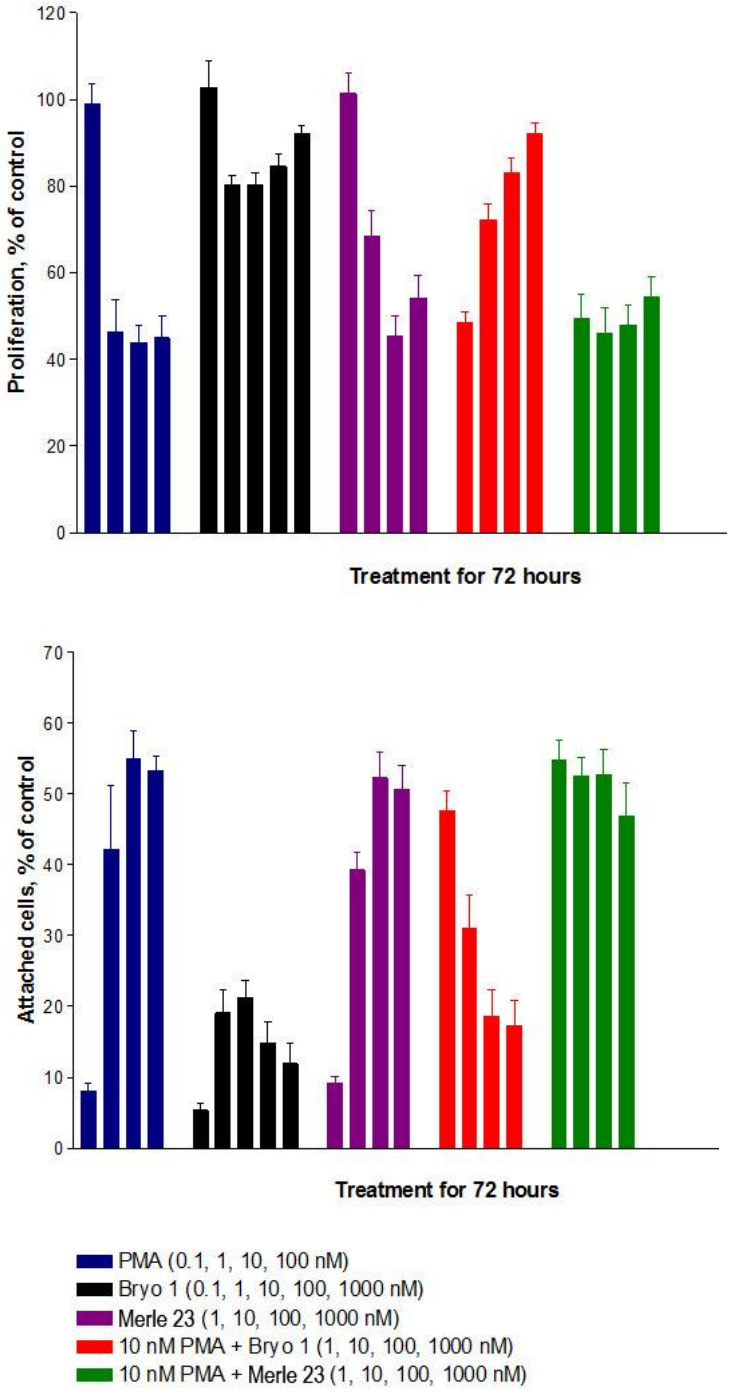


Figure 1.17: Proliferation (top) and attachment (bottom) of U937 cells by **Merle 23**

Wender, Merle 23 would behave like bryostatin 1.

With the information from the biological studies of these analogues, we set out to determine the role of different substitutions on the A and B-rings of the bryostatin 1.

Synthesis of C_{30}-Decarbomethoxy Bryostatin 1

Bryostatin 1 and its simplified analogue Merle 23 differ from each other just at four positions (C_7, C_8, C_7 and C_{13}) on the A- and B-ring region. It is thus proposed that these four positions are responsible for the PMA like activity of Merle 23 as opposed to that of bryostatin 1. In order to determine the substitution crucial for the switch of the biological effect, we decided to systematically remove one group at a time from the bryostatin 1 structure at these four positions and observe the corresponding biological effect. From a synthetic point of view, it seemed most logical to first remove the distant substitution on the B-ring among all four positions. Thus, removal of the carbomethoxy group from the C_{30} position on the B-ring would lead an analogue that would differ from bryostatin 1 just at one position and the role of this substituent could be determined. If the substitution on this positiosn has no effect on the end biological effect, then the role of other position on the A-ring would be investigated.

Retrosynthesis

Figure **1.17** shows retrosynthetic plan for the synthesis of C_{30}-decarbomethoxy bryostatin 1. We envisioned installing the unsaturation on the C_{21} and C_{22} positions through a late stage functionalization of C-ring of a tricyclecycle such as **1.67**. The B-ring of the **1.67** would be constructed via a crucial pyran annulation of β-hydroxyallyl silane

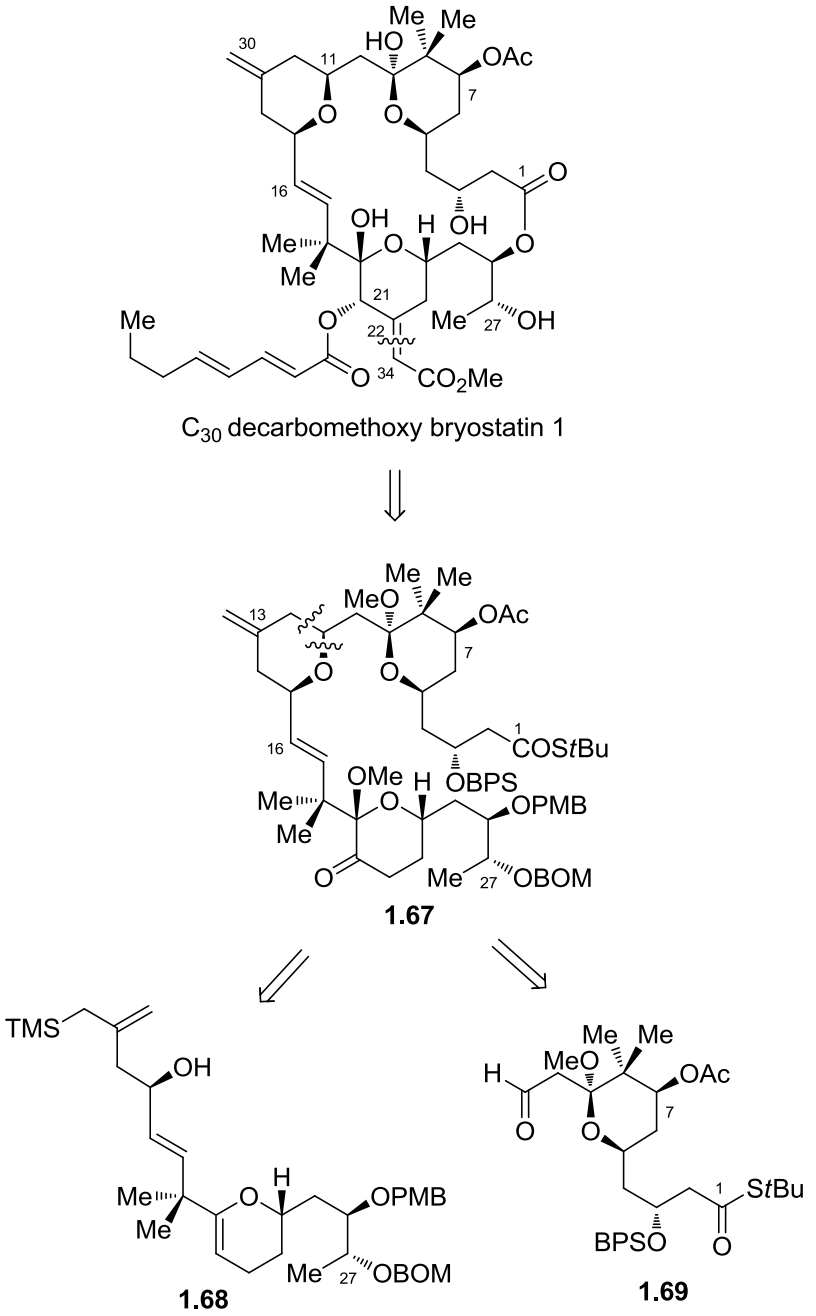


Figure 1.17 Retrosynthesis of C_{30}-decarbomethoxy Bryostatin 1

1.68 with the aldehyde **1.69**. The highly convergent union of these two fragments of almost equal complexity would not only construct the B-ring in a very convergent manner, but would also install the two stereocenters around the B-ring pyran. The flexibility of this methodology is such that the coupling partners could be reversed with A-ring as silane and C-ring as aldehyde. The latter methodology has been successfully applied in the total synthesis of bryostatin analogues mentioned before.[38] The other termins of these pieces would be coupled by Yamaguchi esterification.

Our design of the A-ring aldehyde **1.69** is based on Lewis acid mediated substrate controlled nucleophilic additions in order to create the necessary stereocenters. The A-ring of the bryostatin could be formed by the ketalization of a keto-alcohol derived from an acyclic precursor such as **1.70** (Figure **1.18**). The acyclic fragment **1.70** has a C_3-C_5-C_7 anti stereotriad which allows us to apply our 1,3 chelation controlled addition methodology. Thus, a series of chelation controlled additions of the nucleopliles on either side of the C_5 stereocenter would create the C_3 and C_7 stereoenters. One such nucleophile is stannane **1.71** which when added to the aldehyde **1.72** would not only create the C_7 stereocenter but also install the gem-dimethyl group. This is particularly interesting because most previous bryostatin analogue syntheses are built around a gem-dimethhyl group brought in from a simple starting material. In addition, the use of such a functionalized stannane would make the synthesis of A-ring itself highly convergent. The C_5 stereocenter would be installed by another chelation controlled addition of the thioketene acetal **1.75** on aldehyde **1.74**. The crucial C_5 stereocenter would be installed by using our catalytic asymmetric allylation (CAA) reaction.

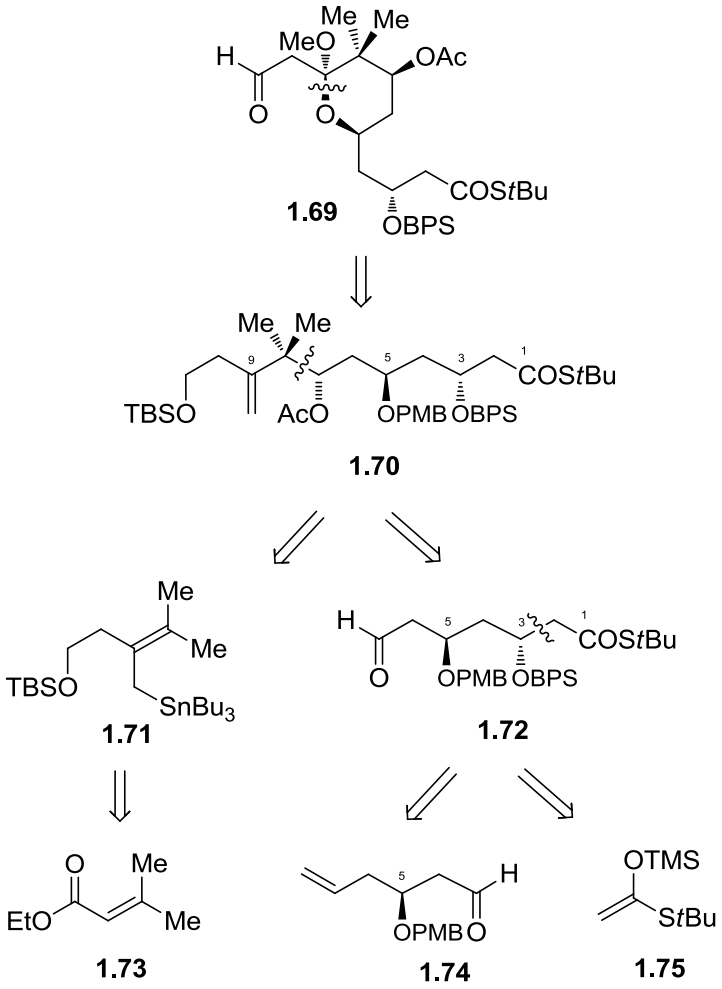


Figure 1.18. Retrosynthesis of A-ring Aldehyde **1.69**

The β-hydroxy allylsilane functionality of the C-ring was envisioned to come from a CAA reaction of trimethylsilylmethallyl stannane on aldehyde **1.76** (Figure **1.19**). The α,β-unsaturated aldehyde would be formed by a HWE olefination of the aldehyde derived from olefin **1.77**. The gem-dimethyl group could be installed by a prenylation of aldehyde **1.78**. A hydroformylation of the olefin **1.79** would install the aldehyde handle. Since compound **1.79** has 1,2-syn and 1,3-anti alcohol stereocenters, it would come

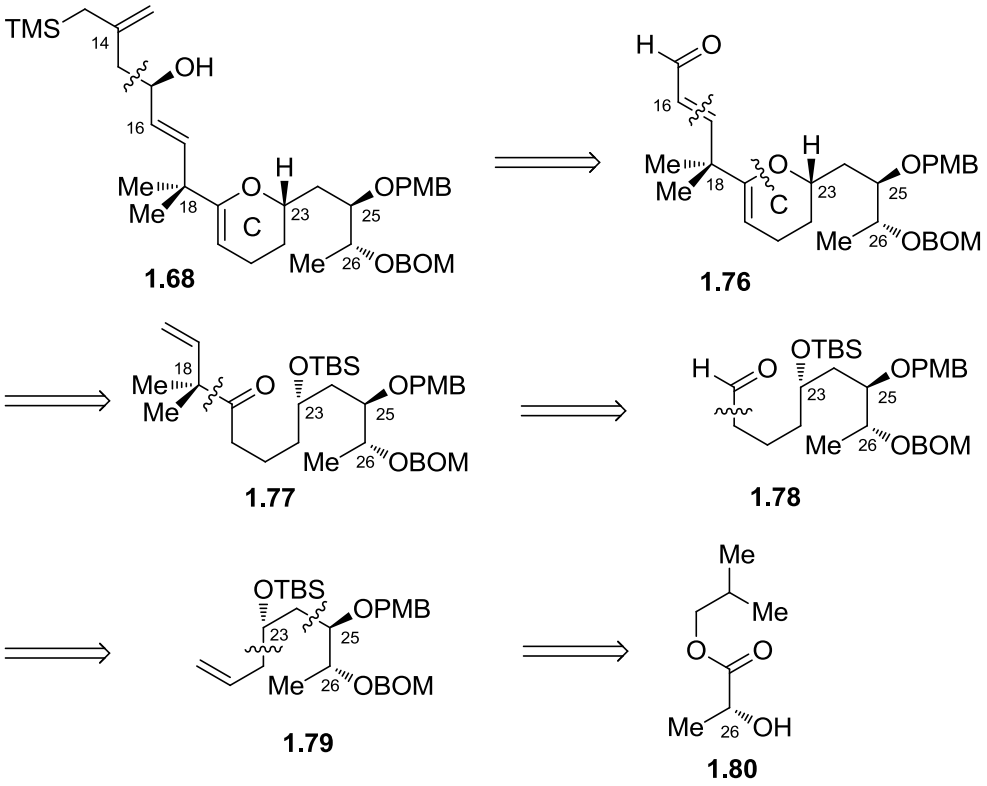


Figure 1.19: Retrosynthesis of C-ring Silane **1.68**

from a series of chelation controlled addition of allylstanne to the aldehyde derived from the commercially available lactate **1.80**. It is important to note that most of the stereocenters on the C-ring would be derived from a single stereocenter present in the lactate **1.80** exhibiting a highly substrate controlled synthesis.

Synthesis of A-ring Aldehyde

The synthesis of the A-ring aldehyde **1.69** commenced with the preparation of C_1-C_7 fragment **1.72** (Figure **1.20**).[41] Monoprotection of the commercially available 1,3-propane diol followed by Swern oxidation generated the aldehyde **1.83**.[42] A catalytic

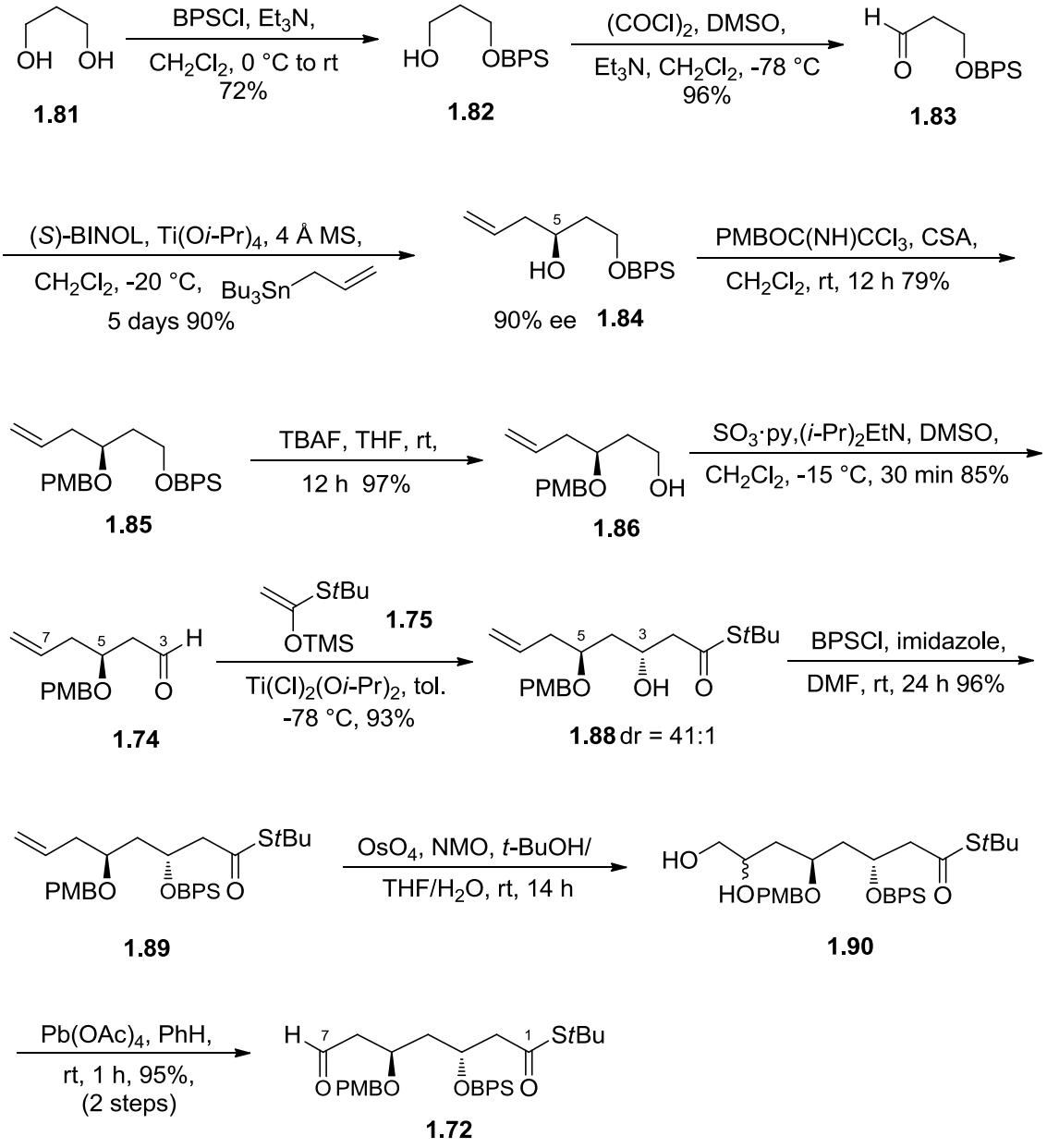


Figure 1.20. Synthesis of C_1-C_7 Fragment

asymmetric allylation reaction on aldehyde **1.83** proceeded with excellent yield and enantioselectivity providing the homoallylic alcohol **1.84**.[43] The newly generated C_5 stereocenter was later used for the chelation controlled addition reactions on both sides to generate the C_3 and C_7 stereocenters. The free alcohol was protected as a PMB ether under acidic conditions as basic condition led to extensive 1,3 migration of the silyl protecting group resulting in an inseparable mixture of products. The BPS protecting group was then removed using TBAF and the resulting alcohol was oxidized to an aldehyde using the Parikh-Doering reaction.[44] It was found that aldehyde **1.74** was prone to β-elimination of the PMB group if old bottles of SO_3•py were used or if the reaction temperature was increased above 0 °C. The aldehyde **1.74** was used next for the first chelation controlled Mukaiyama aldol reaction that would generate the C_3 stereocenter. Use of Lewis acids like $MgBr_2$•OEt_2 or BF_3•OEt_2 resulted in only moderate diastereoselectivity and yield. However the application of 2.5 equivalent of $Ti(Cl)_2(O$*i*-$Pr)_2$ in toluene provided the desired product with high diastereoselectivity. After the successful establishment of the C_3 stereocenter, the resulting alcohol was protected as its BPS ether. A two-step oxidative cleavage of the olefin was found to be superior to ozonolysis and generated aldehyde **1.72** in nearly quantitative yield.

The synthesis of the stannane **1.93** was addressed next (Figure **1.21**). A vinylogous alkylation of the lithium enolate of commercially available ethyl 2,2-dimethylacrylate with TBS protected 2-iodoethanol provided the desired product in good yield. The olefin was then migrated to the more thermodynamically favored α,β-unsaturated position using KO*t*Bu. It was necessary to rigorously exclude oxygen during the isomerization in order to prevent any oxidation of the potassium enolate.[45] Full

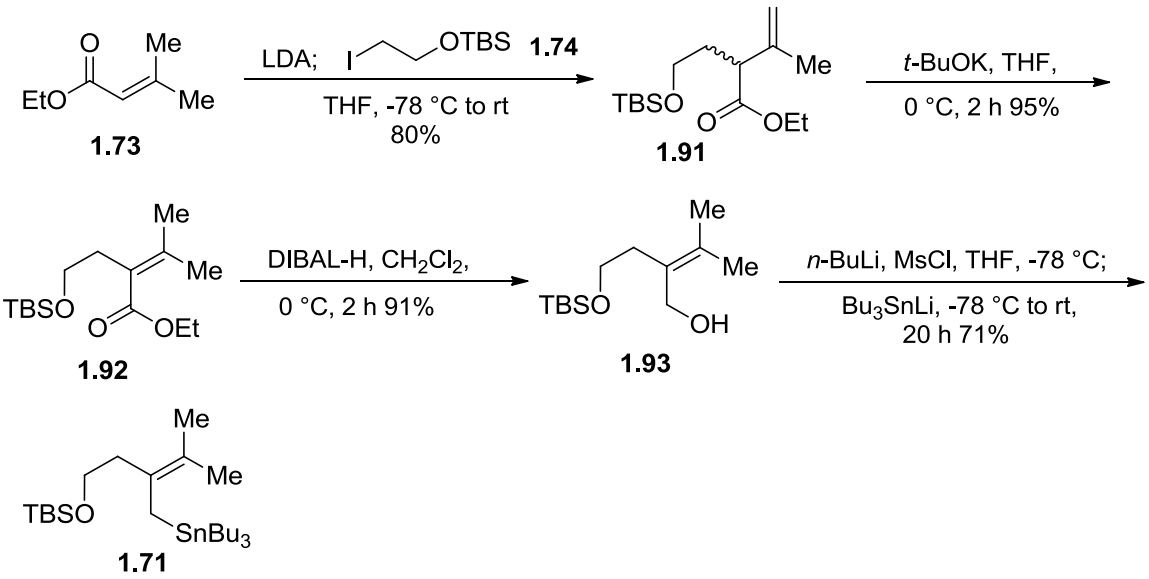


Figure 1.21. Synthesis of Stanne **1.71**

reduction of ester **1.92** using DIBAL-H provided the allylic alcohol **1.93**. Conversion of the alcohol to the corresponding chloride, bromide or mesylate provided the desired products which were unstable for isolation and purification. Thus the conversion of the alcohol into a mesylate at –78°C followed by *in situ* displacement with tributyltinlithiate provided the desired allylstannane **1.71** in a 70% yield.

With aldehyde **1.72** and stannane **1.71** in hand, the crucial chelation controlled addition reaction was attempted (Figure **1.22**). According to our previous observations, such an addition reaction would need a strong Lewis acid.[46] Thus activation of the aldehyde **1.72** by precomplexation with Me_2AlCl in toluene at –78 °C followed by slow addition of stannane **1.71** provided the coupled product in 76% yield as a single isomer as observed by ^1H and ^{13}C NMR spectroscopy.[47] It was extremely important to maintain the chelation time at 5 min in order to avoid the formation of a secondary alcohol byproduct formed by the transfer of a methyl transfer from the Me_2AlCl to the aldehyde.

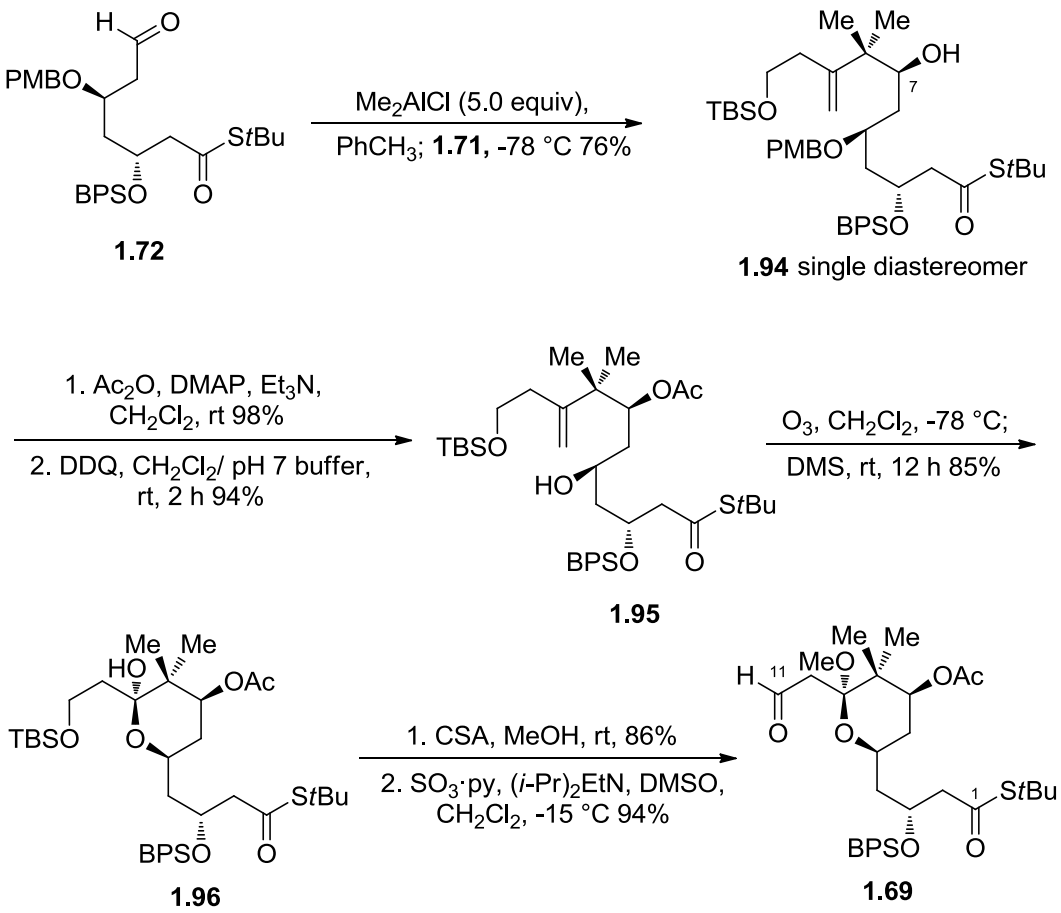
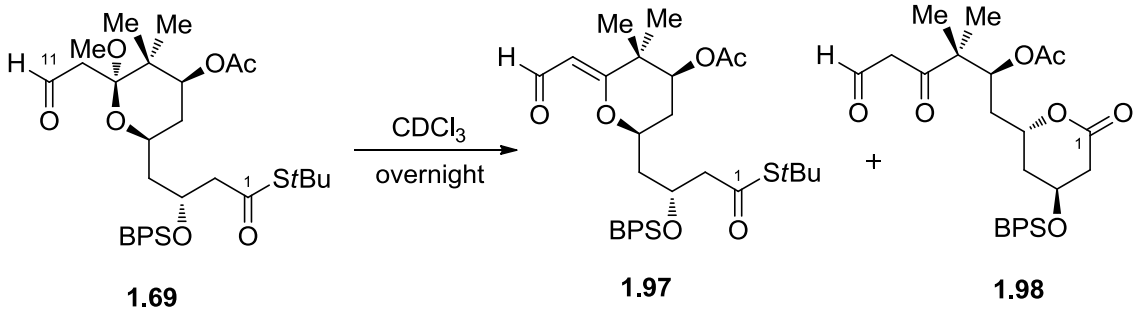


Figure 1.22: Completion of the A-ring Aldehyde **1.69**

After the successful installation of the gem-dimethyl group as well as the C_7 stereocenter, the newly formed alcohol was converted into the acetate which is present in the natural product. Removal of the PMB group under oxidative conditions using buffered DDQ provided the alcohol. The alcohol proved to be unstable for long term storage as it underwent lactonization with the terminal thioester. Ozonolysis of the olefin followed by reductive work-up yielded the hemiketal **1.96** in 91% yield. The hemiketal **1.96** was found prone to equilibrate to the corresponding keto-alcohol. Such equilibration

with the open chain compound was more deleterious in the present context due to lactonization of the resulting alcohol with the thiolester carbonyl. Thus, treatment of the hemiketal with acidic methanol converted the unstable hemiketal into the more stable methylketal as well as removing the TBS group in a single operation. The primary alcohol was then subsequently oxidized to aldehyde **1.69** by using Parikh-Doering conditions to generate the A-ring subunit **1.69** in a 94% yield.

During the preparation of the aldehyde **1.69**, it was observed that the aldehyde decomposed significantly when it was not purified right after reaction workup (Figure **1.23**). Moreover, overnight NMR using $CDCl_3$ also led to the decomposition of the material. Mass spectrometric analysis of the byproduct indicated the presence α,β-unsaturated aldehyde **1.97** as well as the lactone **1.98**. The presence of such byproducts was not too surprising since $CDCl_3$ is known to contain small amount of DCl/D_2O which could catalyze the hydrolysis/elimination of the methoxy group. No such decomposition was observed when the aldehyde was purified immediately after the reaction and $CDCl_3$ was neutralized by passing through activated Al_2O_3. However, attempted conversion of **1.97** into the desired product **1.69** using acidic methanol was unsuccessful.



1.69 **1.97** **1.98**

Figure 1.23. Some of the Byproducts from Decomposition of Aldehyde **1.69**

Such acid lability of the methyl ketal was very concerning regarding the potential use of aldehyde **1.69** in a pyran annulation reaction. In order to examine if the aldehyde **1.69** would survive in a Lewis acid mediated reaction, we subjected it to a pyran annulation reaction with the simple *β*-hydroxyallyl silane **1.99**. To our delight, the reaction provided the bicyclic compound in 85% yield as a single diastereomer (Figure **1.24**). Diagnostic NOE transfers from the C_{11} proton to the C_{15} and C_9 methoxy protons proved the expected stereochemistry about the newly formed B-ring. This reaction thus confirmed the validity of the aldehyde **1.69** as a suitable substrate for the pyran annulation with an even more complex C-ring β-hydroxy allylsilane **1.68** as proposed in the retrosynthetic analysis. In addition, the bicyclic compound **1.100** itself could be further functionalized and used in the synthesis of the bryostatins and their analogues.

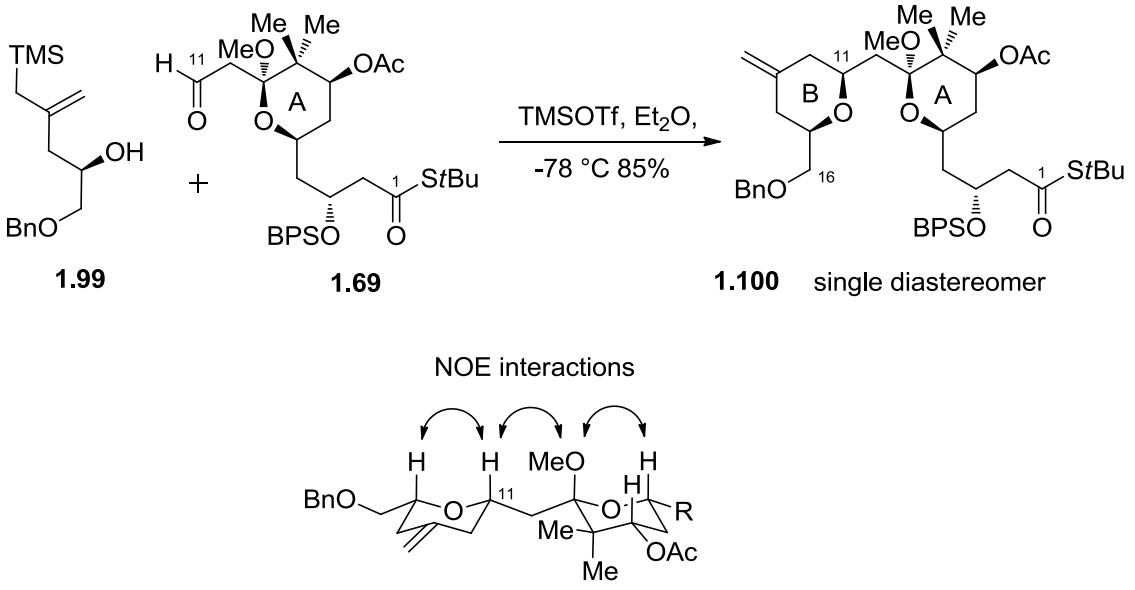


Figure 1.24 Model Pyran Annulation and Synthesis of A-B-ring

Synthesis of the C-Ring Silane

The route for preparation of the olefin **1.77** needed for the C-ring was developed by Dr. Anh Truong and scaled up for the present synthesis by another graduate student, Jeffrey C. Stephens from our group (Figure **1.25**).[37] Briefly, a BOM protection of commercially available *R*-isobutyl lactate followed by half reduction of the ester using DIBAL-H provided the aldehyde **1.102** in excellent yield. A 1,2-chelation controlled allylation[48] installed the C_{25} stereocenter providing a single alcohol isomer and the resulting alcohol was protected as a PMB ether. Ozonolysis of the olefin furnished the aldehyde **1.105** which was used for a 1,3 chelation[49] controlled allylation to set the C_{23} stereocenter. The allylation provided a 5:1 mixture of diastereomeric alcohols in quantitative yield in favor of the desired isomer. The alcohol isomers were found to be inseparable. However, when the alcohol was protected with TBS group, the mixture of alcohols could be easily separated using silica gel flash column chromatography. Thus both C_{25} and C_{23} stereocenters were installed using substrate mediated chelation controlled additions reactions using C_{27} stereocenter One carbon homologation of the olefin **1.79** using Buchwald hydroformylation[50] provided the aldehyde **1.78** as a single linear formylated product.The gem-dimethyl group was installed by the addition of a prenyl indium reagent to the corresponding aldehyde which provided an inconsequential mixture of alcohol diastereomers. Oxidation of the alcohols using Parikh-Doering conditions completed the synthesis of C_{16}-C_{27} fragment.[44] Thus the synthesis of the C_{16}-C_{27} fragment was completed in ten linear steps from commercially available *R*-isobutyl lactate **1.80**.

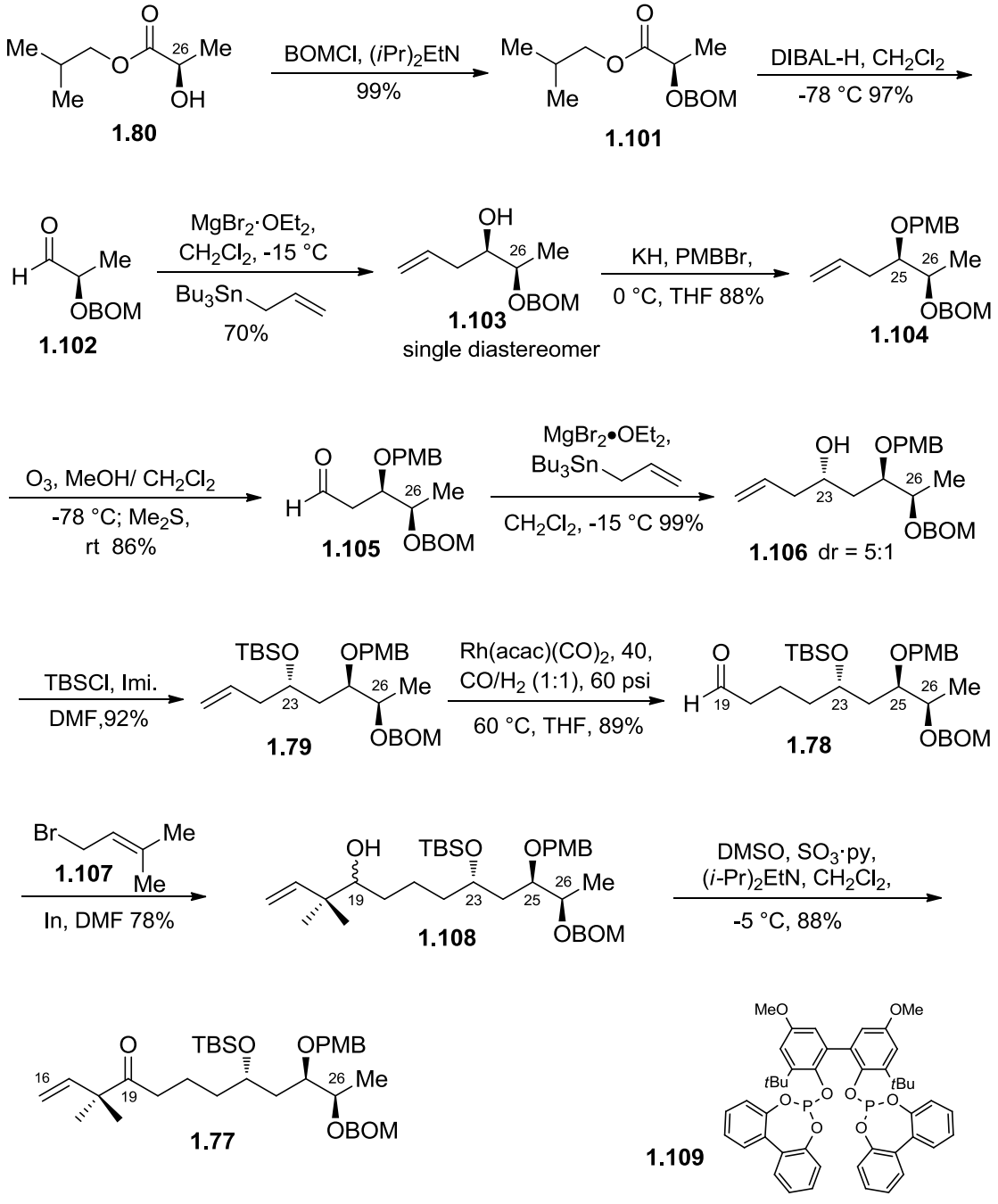


Figure 1.25. Synthesis of C_{16}-C_{27} Fragment **1.77**

Further functionalizaton of the olefin **1.77** involved ozonolysis with reductive workup providing aldehyde **1.110**, which was found to be unstable for chromatographic isolation (Figure **1.26**). Therefore the crude aldehyde **1.110** was subjected to a Horner-Wadsworth-Emmons olefination with a thiolester phosphate reagent which resulted in the formation of a single geometric isomer of the α,β-unsaturated thioester **1.111** in good yield.[54] Removal of the TBS group followed by dehydrative cyclization provided the

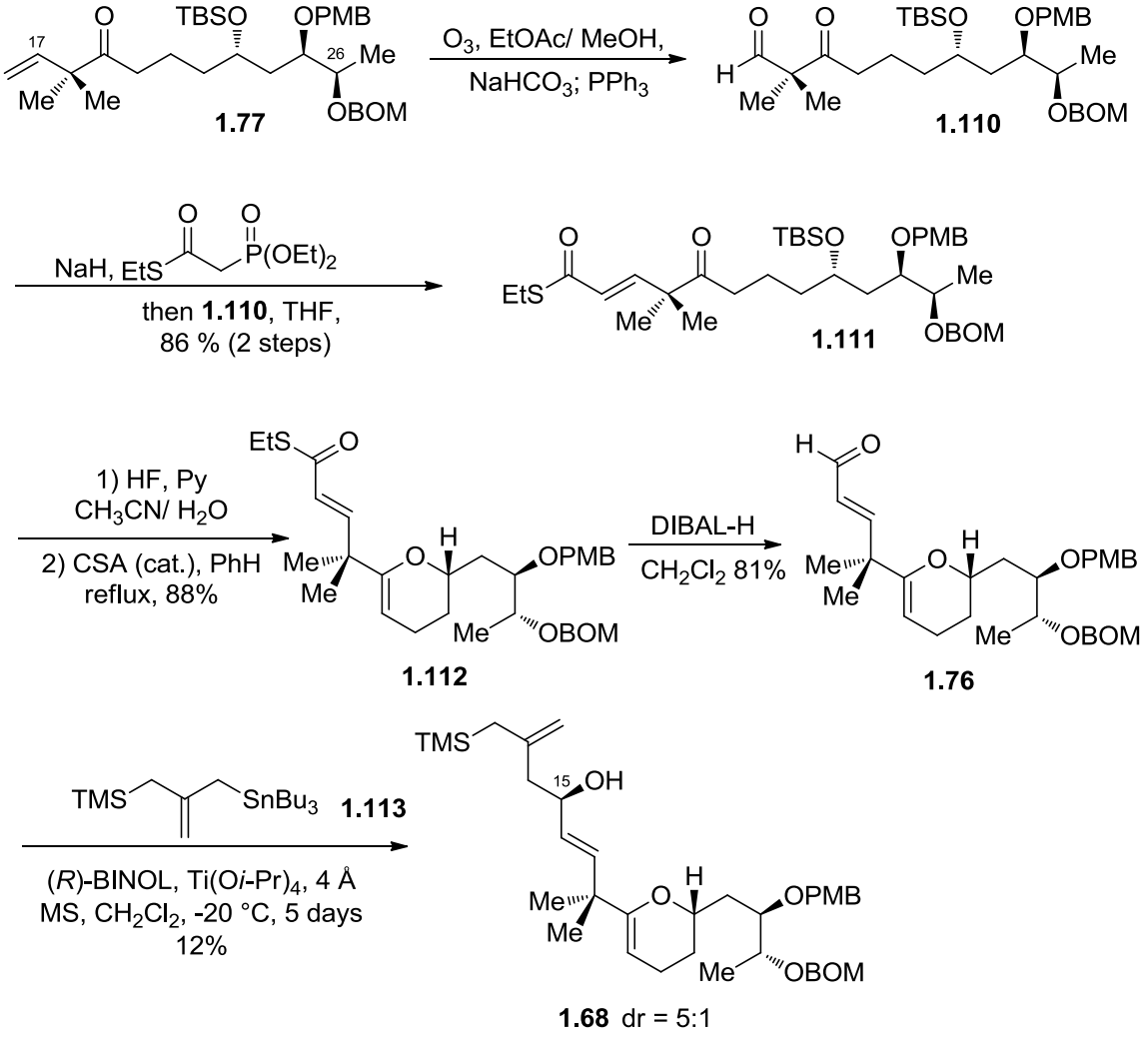


Figure 1.26. Synthesis of C-ring Silane **1.68**

glycal **1.112**. It should be noted that long term storage of the glycal in benzene resulted in partial decomposition resulting from the hydration of the dihydropyran. However, such decomposition could be avoided if the glycal was stored neat. A half reduction of the thioester using DIBAL-H provided the aldehyde **1.76** in good yield.

With aldehyde **1.76** in hand, installation of the β-hydroxyallyl silane functionality was attempted. This involved a CAA reaction of aldehyde **1.26** with trimethylsilylmethylallyl stannane **1.113**.[52] Although successfully used by Dr. Anh Truong in the synthesis of simple bryopyran analogues, this CAA reaction was found to be poorly reproducible. Even with the use of a super-stoichiometric amount of the catalyst, the reaction provided only 5:1 mixture of diastereomers at C_{15} in a very low yield. We believe that the low reactivity of this aldehyde in CAA reaction is both steric and electronic in origin. The more delocalized α,β-unsaturated aldehydes are typically less reactive towards the nucleophilic addition of stannane reagents **1.111** in CAA. This particular aldehyde is comparatively more complex and much more sterically hindered for both the activation by the catalyst and addition of the nucleophile in the CAA reaction.

As attempts to form the silane **1.68** in a stereoselective manner were unsuccessful, we focused our attention towards an alternative solution (Figure **1.27**). One potential way to this solve this problem would be to set the C_{15} alcohol stereocenter by the reduction of the corresponding ketone. Such α,β-unsaturated ketones are known to be good substrates for the CBS reduction or Noyori's BINAL-H reduction. In order to prepare the ketone, the aldehyde **1.76** was heated at reflux with trimethylsilyl methylallyl stannane in toluene which provided a 1:1 mixture of diastereomeric alcohols at C_{15}. It is interesting to note

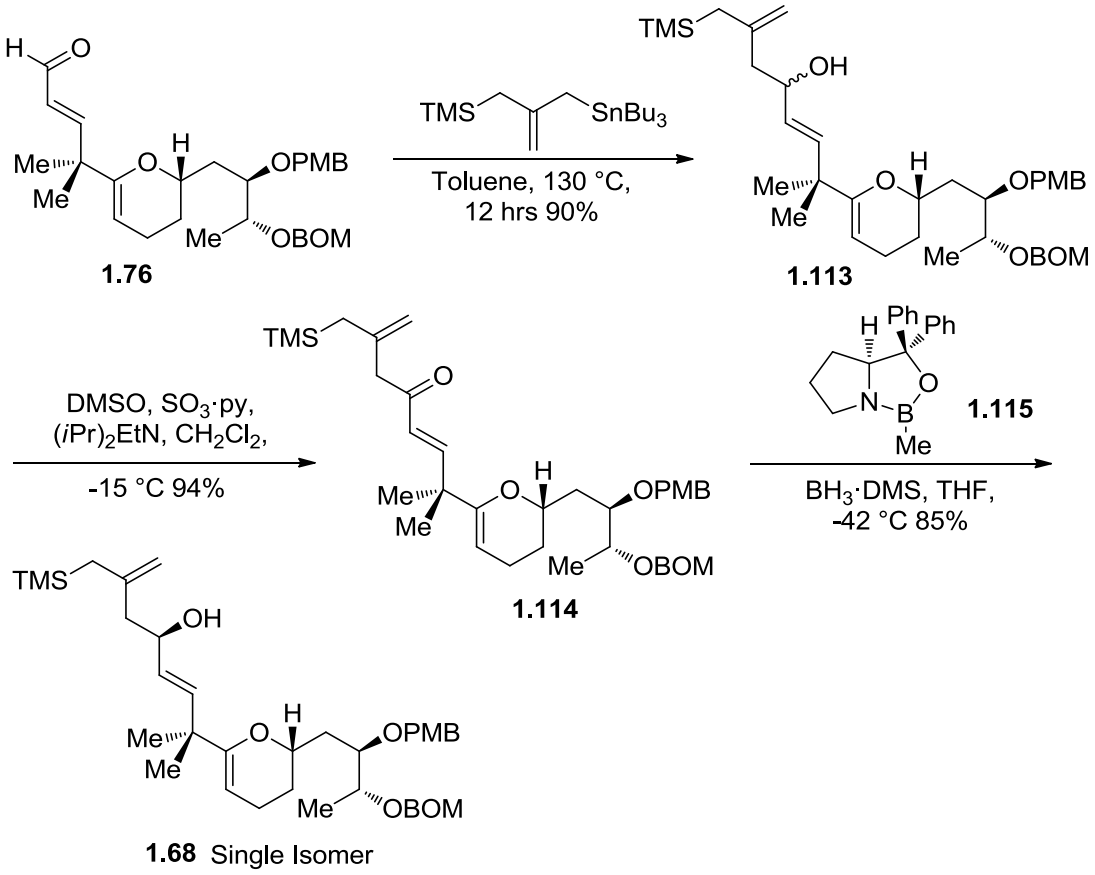


Figure 1.27. Alternative Synthesis of C-ring Silane **1.68**

that the relatively unstable glycal was found to be robust enough for this reaction. The diastereomeric mixture of alcohol was oxidized to the corresponding ketone using a Parikh-Doering oxidation. As expected, the ketone **1.114** was found not to be very stable for long term storage. It was also necessary to immediately purify the ketone right after the reaction workup. The reduction of the ketone was first attempted with Noyori's *R*-BINAL-H.[53]

Although the reaction provided the alcohol in good yield, the diastereoselectivity was only 3:1 at -78 °C. The selectivity could be increased to 5:1 at -100°C, but the yield

dropped to 50%. Moreover, it was found to be really difficult to keep the reaction temperature at -100 °C even for few h using the conventional cooling bath. With little success using the BINAL-H reagent, application of Corey-Bakchi-Shibata (CBS) reagent was attempted next.[54] However the conventional method of running the CBS reduction by mixing the ketone with the CBS reagent and BH_3•DMS at -78 °C provided a complex mixture of products presumably due to the hydroboration and/or reduction of the olefins. However, when the reaction was performed sequentially by first pre-complexing the BH_3•DMS with the CBS reagent at 0 °C followed by the slow addition of the ketone at -40 °C, the reaction provided the desired alcohol as a single diastereomer in good yield. The absolute stereochemistry at the C_{15} position was determined using Mosher ester analysis.[55] This oxidation-reduction sequence easily provided multigram quantities of the silane **1.68** for coupling with aldehyde **1.69**.

Completion of C_{30}-Decarbomethoxy Bryostatin 1 (Merle 28)

With both fragments in hand, the A-ring aldehyde **1.69** and C-ring silane **1.68** were subjected to the crucial pyran annulation reaction (Figure **1.28**). This reaction provided the desired tricyclic compound **1.115** in a 58% yield. Although the yield was moderate, this advanced pyran annulation furnished the tricyclic carbon skeleton of the bryostatin analogue. The majority of the byproduct was O-TMS protected silane and unreacted A-ring aldehyde which could be recovered without appreciable loss. The O-TMS byproduct could be easily converted into the desired silane **1.69** by stirring with PPTS/MeOH at 0 °C. It should be pointed out that monitoring this pyran annulation reaction using TLC was found to be misleading since the reaction turned dark purple in

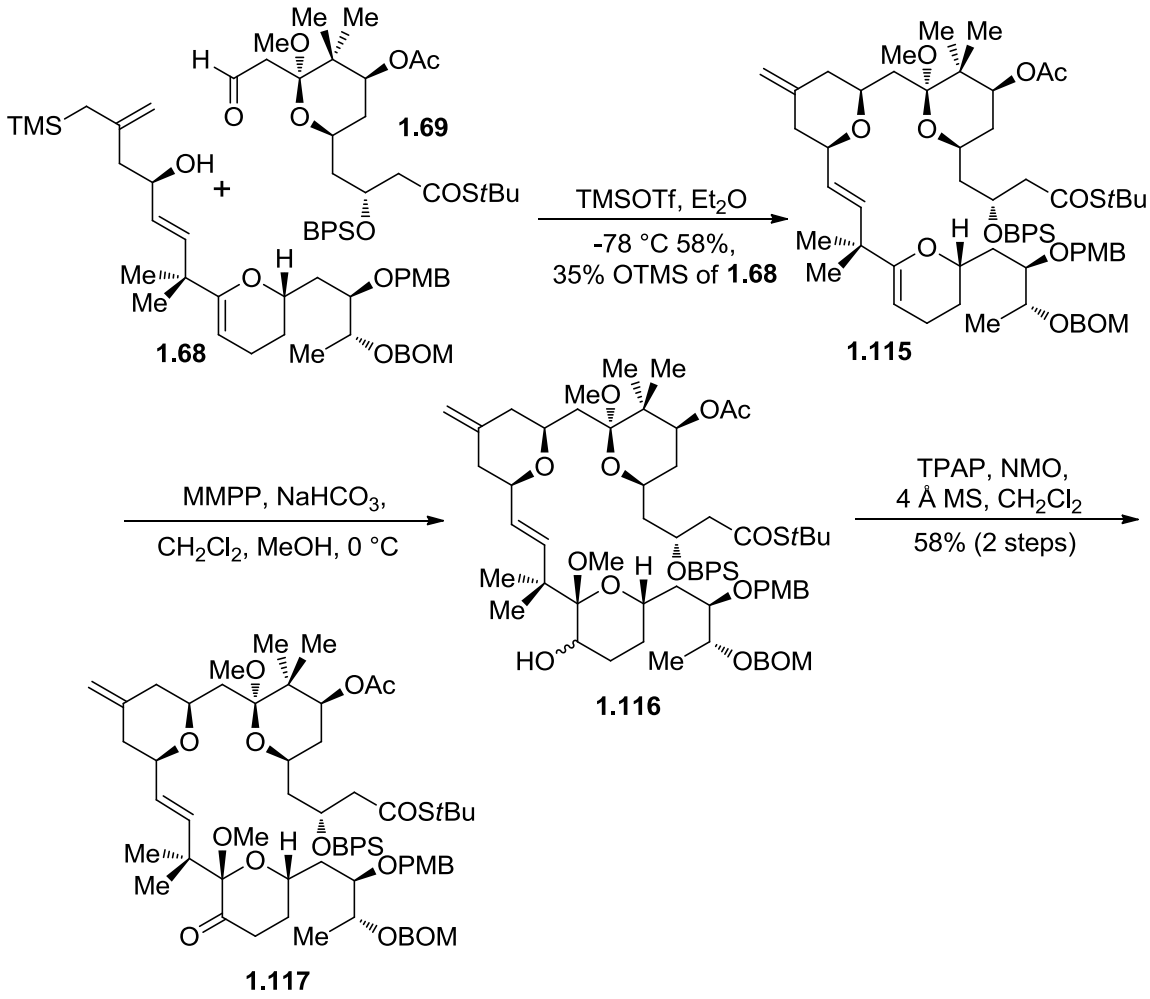


Figure 1.28. Coupling of A- and C-rings Using Pyran Annulation

the TLC spotter and decomposition took place. In addition, it is necessary to run the pyran annulation under anhydrous condition. The presence of any moisture leads to the purple colored reaction mixture and low yields.

Because of the moderate yield of the pyran annulation reaction, several conditions were examined to improve the yield. Increase of temperature, increase/decrease of reaction concentration, and precomplexation of the aldehyde with TMSOTf did not have

any positive impact on the outcome of the reaction. Use of other Lewis acids such as TBSOTf or TESOTf did not provide any product. Since the aldehyde **1.69** provided thebispyran **1.100** in high yield with a model silane (Figure **1.24**), the moderate yield of this reaction could be attributed to the relatively complex nature of silane **1.68** making its accessibility to aldehyde **1.69** difficult due to severe steric congestion around the aldehyde.

With the tricyclic carbon skeleton of the analogue in hand, we turned our attention towards the functionalization of the C-ring (Figure **1.29**). This involved the chemoselective epoxidation of the glycal using MMPP and *in situ* opening of it by methanol to provide methoxy alcohol **1.116**. Attempts to carry out this transformation using *m*-CPBA gave low yield presumably due to the oxidation of the thioester sulfur. The inconsequential mixture of crude alcohols was then oxidized using TPAP/NMO providing the ketone **1.117**.[56] Further functionalization of the C- ring required the installation of the α,β-unsaturated methyl ester at the C_{21} position. This was accomplished by a two step process involving an aldol reaction of the ketone with methylglyoxylate followed by its dehydration (Figure **1.29**). Initial experiments showed that three equivalents of LDA were needed for the reaction to go to completion. Surprisingly, in addition to the desired aldol adduct **1.119**, the reaction also provided the bis-adduct **1.118** in which the aldol reaction took place at C_7 acetate. This side reaction at the C_7 acetate could be avoided by using only one equivalent of base but the yield of desired product did not increase above 50%. However, the unreacted ketone starting material was recovered quantitatively and was recycled. Elimination of the aldol adduct was carried out using carbonyl diimidazole (CDI) which provided the desired α,β-

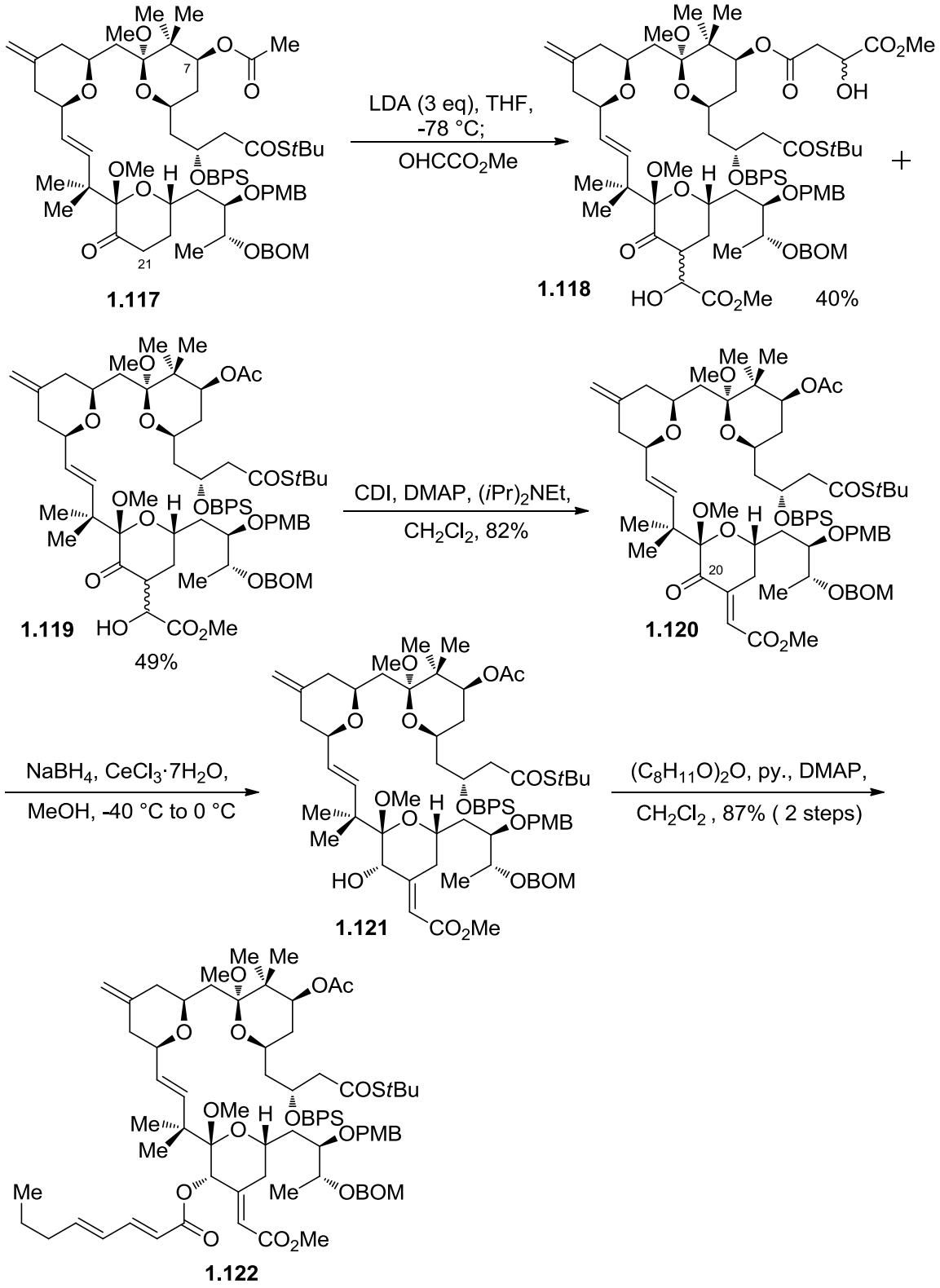


Figure 1.29 Functionalization of the C-ring

unsaturated ester as single isomer. It is interesting to note that any C-ring intermediates that have this type of keto-ester functionality were bright yellow in color (*vide infra*) and all of them proved to be unstable for long term storage. However, the decomposition could be reduced by storing the olefin **1.120** in the dark. Luch reduction[57] of ketone **1.120** set the C_{20} stereocenter providing the alcohol **1.121** which also proved to be unstable for chromatographic separation. The bryostatin side chain was thus installed by an immediate esterification of the alcohol with the octadienoic anhydride.

With all the necessary functional groups present in compound **1.122**, the next step would be the preparation of the seco acid for macrolactonization (Figure **1.30**). Unfortunately the hydrolysis of the thioester to reveal the carboxylic acid was found to be problematic. Use of conventional procedures employing $LiOH/H_2O_2$ resulted in a complex mixture of products due to competitive hydrolysis of various esters. Other conditions such as basic aq. $AgNO_3$ partially hydrolyzed and/or eliminated the C_9 methylketal. Use of $Hg(OCOCF_3)_2$ resulted in the oxymercuration of the various olefins leading to a complex mixture. In order to prevent the loss of this valuable material while attempting hydrolysis reaction, a model study was devised with a simple thioester (Figure **1.31**). The thioester **1.89** was inert to hydrolysis with $LiOH/H_2O_2$ even at room temperature for several h. In contrast, when the thiolester **1.88** with a free alcohol group at the C_3 position was subjected to the same reaction condition, the reaction was complete within 15 min. The rapidity of this hydrolysis is likely because of the lack of steric hindrance imposed by BPS group and due to the activation of the ester carbonyl by an intramolecular H-bonding with the β-alcohol. When these reaction conditions were applied to the real substrate **1.124**, we were pleased to find that the hydrolysis occurred

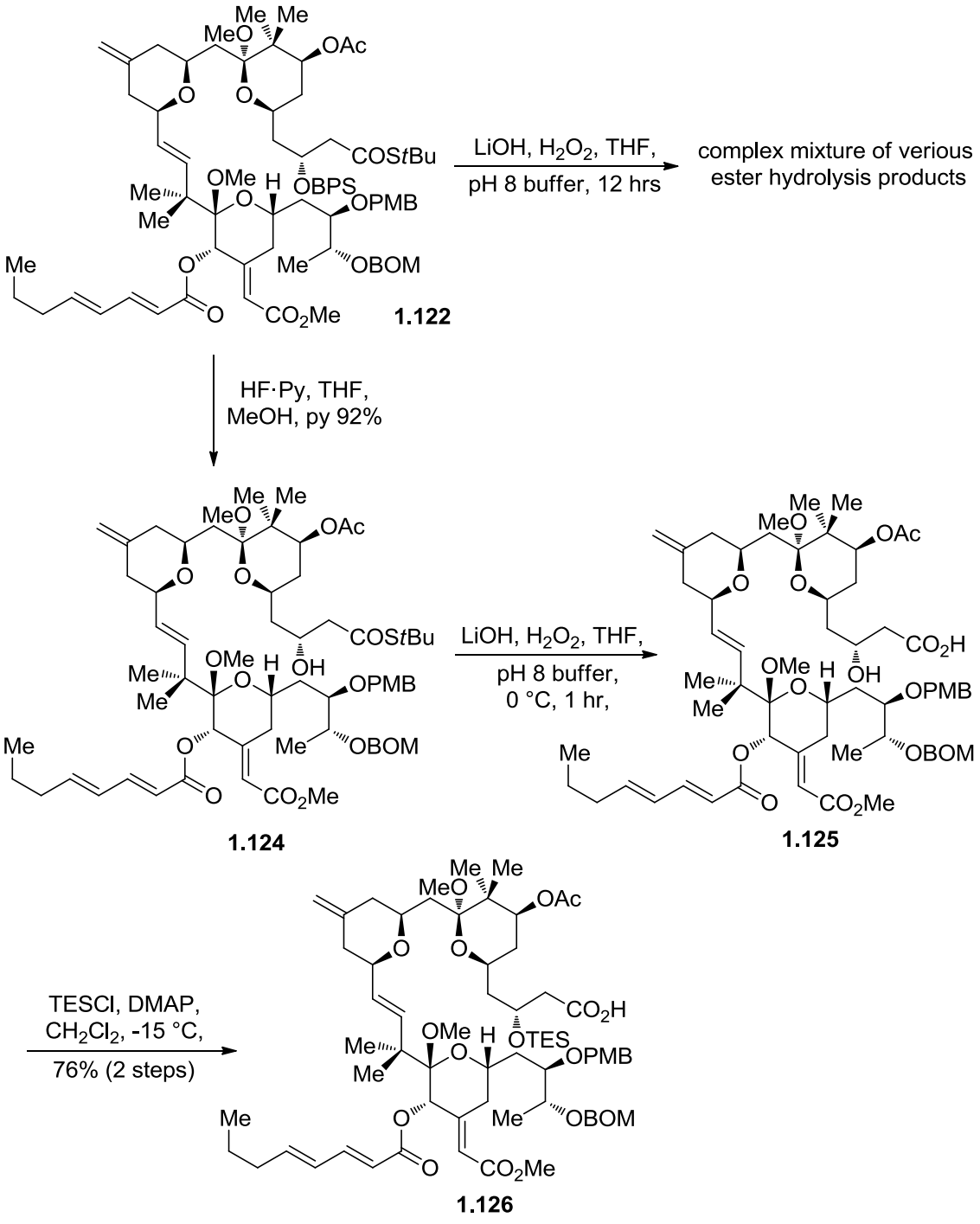


Figure 1.30. Synthesis of the Carboxylic Acid

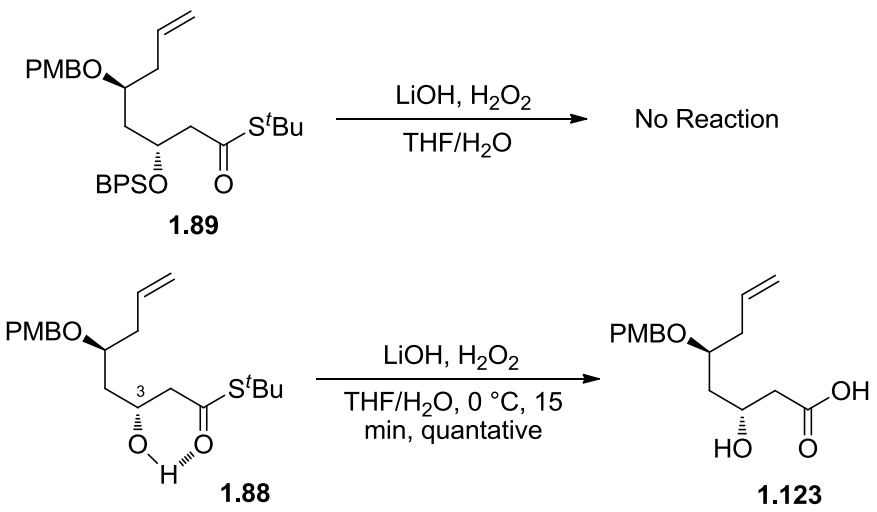


Figure 1.31. Model Hydrolysis of Thioester

smoothly providing the hydroxyacid **1.125**. Stirring of the compound **1.125** with TESCl protected both alcohol and the carboxylic acid which upon workup and column chromatography removed the TES ester providing the carboxylic acid **1.126** in good yield.

In order to prepare the seco acid, the PMB group was removed using DDQ (Figure **1.32**). This reaction needed to be monitored carefully since DDQ competitively deprotected the TES group. If the reaction is allowed to go longer than 2 h at 0 °C, there was complete TES deprotection resulting into the formation of an extremely polar dihydroxy acid which was impossible to separate from the DDQ byproduct. With the seco acid **1.127** in hand, the macrocycle was closed using the Yamaguchi reaction providing the protected analogue **1.128**.[61] A global deprotection using $LiBF_4$ hydrolyzed two methylketals, and removed a BOM and a TES group providing the C_{30} decarbomethoxy bryostatin 1 or Merle 28.[62]

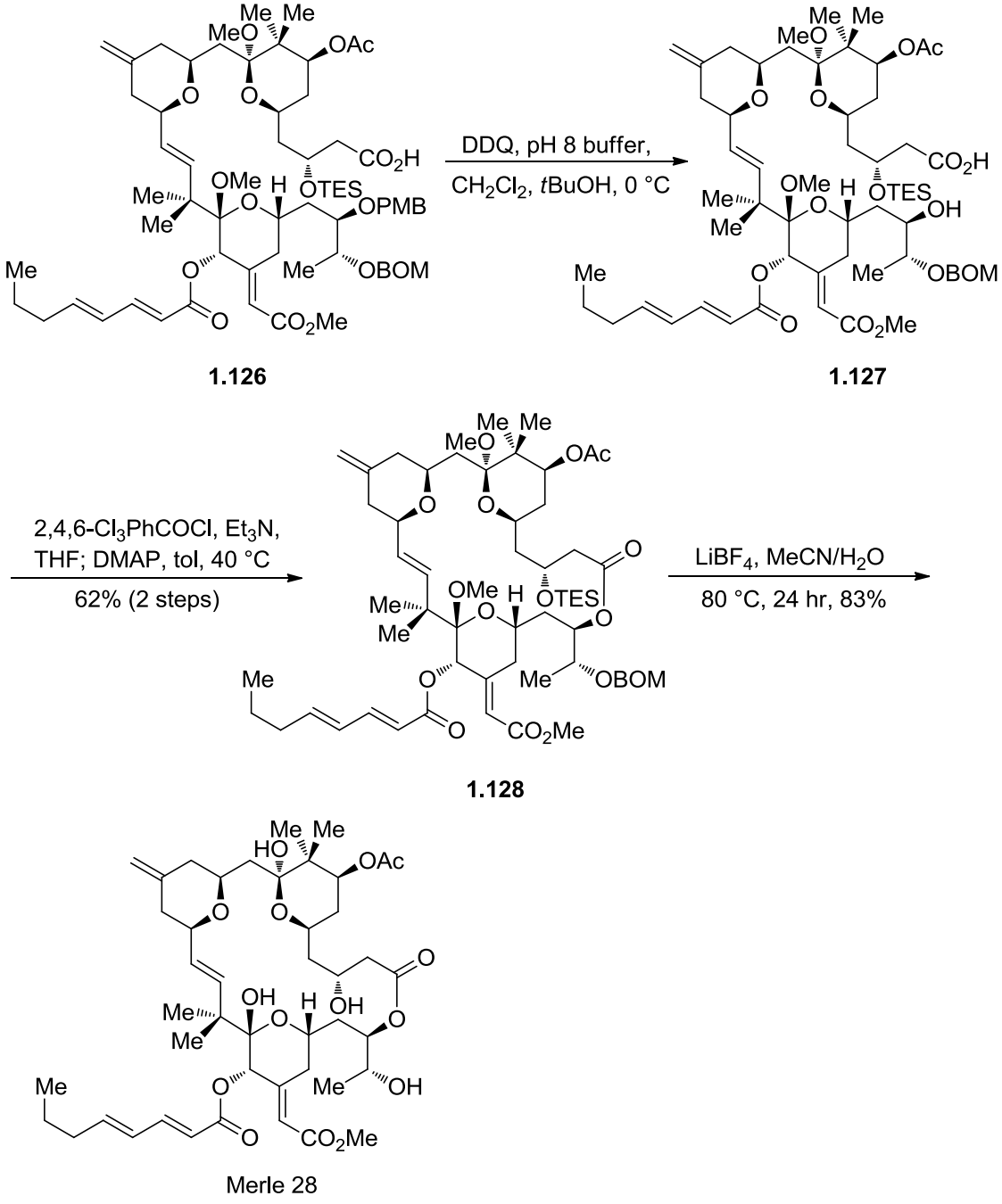


Figure 1.32. Completion of C_{30}-decarbomethoxy Bryostatin 1

Biological Evaluation of C_{30}-Decarbomethoxy Bryostatin 1

The biological evaluation of Merle 28 was carried out by Dr. Peter M. Blumberg at National Cancer Institute, NIH at Bethesda, Maryland.

1. Binding affinity of Merle 28. The first assay involved the determination of potency of Merle 28 by measuring its binding affinity to PKC-α. This assay is an important first step in determining whether simplified analogues retain similar potency to that of the natural product or not. The potency of Merle 28 was tested by measuring its binding ability to the PKC-α expressed as inhibitory dissociation constant (K*i*). The smaller the dissociation constant, the higher the binding affinity and the higher the potency. The determination of K*i* was done by a competitive binding assay of Merele 28 with the bound [20-3H]phorbol 12,13-dibutyrate (PDBu) from mouse recombinant isozyme PKC-α. The assay revealed that Merle 28 had higher binding affinity (K*i* = 0.52 ± 0.06 nM average of three experiments) to that of bryostatin 1 (K*i* = 1.35 nM). This result suggested that C_{30} carbomethoxy group of bryostatin 1 has a little less effect on the binding affinity with PKC-α.

2. Determination of the biological function. Bryostatin is one of the high affinity ligands for PKC. Other PKC ligands are phorbol esters and natural activators such as DAGs. Although phorbol esers are high affinity PKC ligands, in contrast to bryostatins, they are tumor promoters. Thus, the high binding affinity of a ligand does not necessarily reflect anything about biological function. Therefore, it is extremely important to differentiate various ligands of PKC in terms of their function. The biological function of these ligands can be determined by observing their behavior towards certain cells using various assays. Two such assays are the proliferation and attachment of U937 human

lymphoma cells which are used to determine whether a PKC ligand is likely a tumor promoter or not. These assays are used to differentiate the functional antagonism exhibited by the various PKC ligands such as bryostatin and phorbol ester. In the attachment assay, the tumor promoting PMA induces attachment of U937 cells in a dose dependent manner while nontumor promoting bryostatin 1 shows a much diminished effect. In addition, when both bryostatin and PMA are administered together, bryostatin 1 antagonizes and blocks the effect of the phorbol ester in a dose dependent manner.

When the analogue Merle 28 was subjected to the attachment assay (Figure **1.33**), it induced attachment similar to that of bryostatin 1 and different from that of PMA. Moreover, bryostatin shows a unique biphasic phenomenon with is highest attachment at

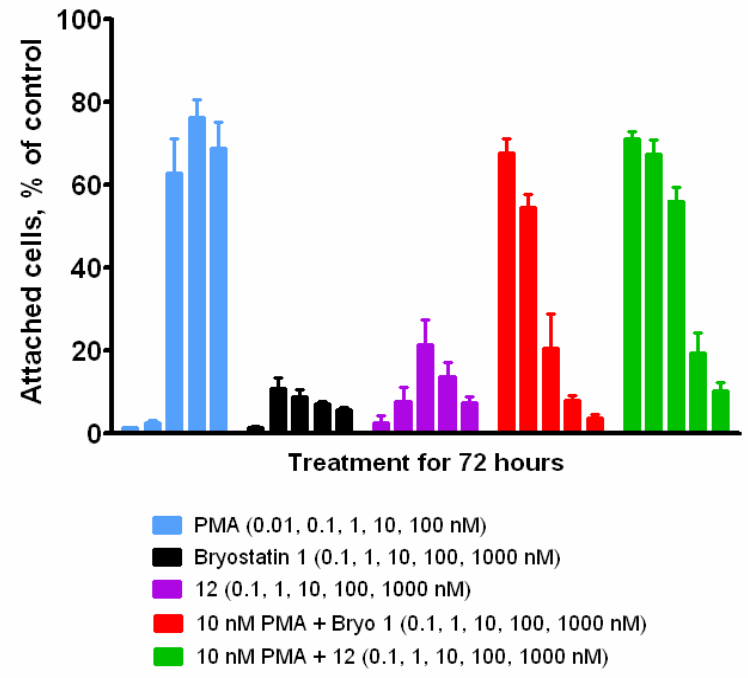


Figure 1.33. Attachment of U937 Cells by Merle 28

moderate concentration and lower attachment at lowest and highest concentrations. It is clear from the graph that Merle 28 displays the dose-dependent biphasic response characteristic of bryostatin 1. Moreover, when Merle 28 and PMA were administered together, Merle 28 blocked the effect of PMA exactly similar to that of bryostatin 1.Thus, from the attachment assay, it can be concluded that Merle 28 behaves like bryostatin 1 and is a functional antagonist of PMA.

In the proliferation assay (Figure **1.34**.), PMA is strongly antiproliferative whereas bryostatin is weakly antiproliferative. Similar to the attachment assay, bryostatin 1 blocks the effect of PMA in a dose dependent manner when both agents are tested together. From this assay, it is clear that the Merle 28 is very similar to bryostatin 1.

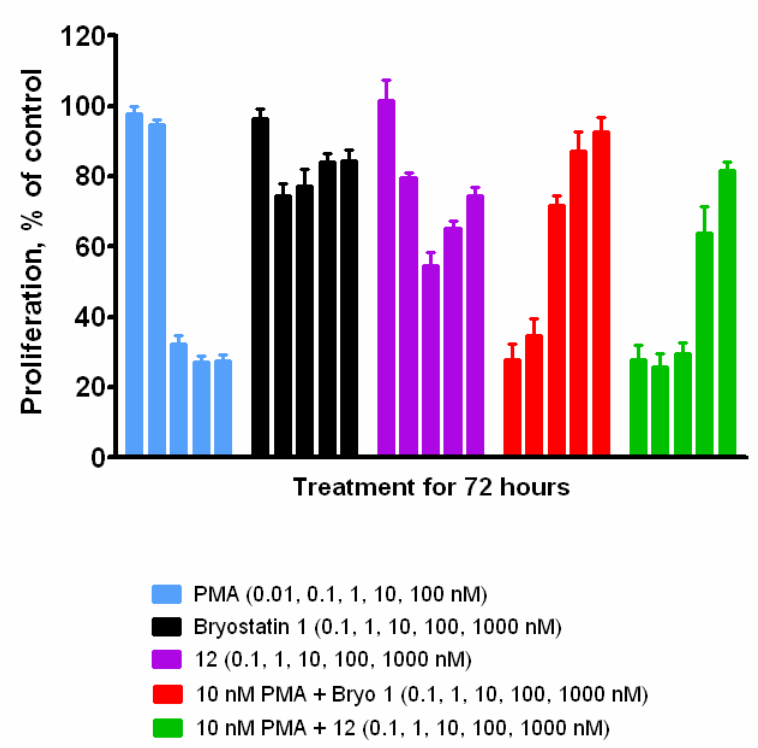


Figure 1.34. Proliferation of U937 Cells by Merle 28

Moreover, both bryostatin and Merle 28 share similar biphasic behavior which is absent with PMA. In addition, Merle 28 blocks the effect of the PMA in a dose dependent manner which is a characteristic feature of bryostatin. Even though Merle 28 has binding affinity comparable to that of bryostatin 1, it lost some potency compared to bryostatin 1 in both the cell attachment and proliferation assays. This can be observed by slight increase in the attachment, decrease in the proliferation as well as decreased blockage of the effect of PMA by Merle 28 compared to that by bryostain 1.

From study of the biological evaluation of Merle 28, it is clear that the absence of C_{30} carbomethoxy group does not decrease the binding affinity towards PKCα. In terms of function, this group is not necessary for bryostatin like biological activity. Thus, it can be concluded from these two assays that Merle 28, which is a very close structural analogue of bryostatin, is also a functional analogue of it. Since the C_{30} carbomethoxy group is not needed for bryostatin like biological activity, this reduces the complexity of analogue design by one level. Thus Merle 28 can act as lead in preparing simplified B-ring analogues that retain bryostatin 1 like biological activity.

As mentioned earlier, Merle 23 chemically differs from bryostatin 1 in four positions but biologically behaves like PMA. The effect of one of such positions, the C_{30} carbomethoxy group, is addressed with the synthesis and biological evaluation of Merle 28.[60] Since Merle 28 differs from Merle 23 in three positions on the A-ring (Figure **1.35**), these three position must be largly responsible for the bryo like biological activity of the former as opposed to the latter one. The effect of the C_7 acetate group was studied by another graduate student Wei Li from our group who prepared bryostatin analogue Merle 27 with an acetate in the C_7 position (Figure **1.36**) [61] The biological study (Figure **1.37**)

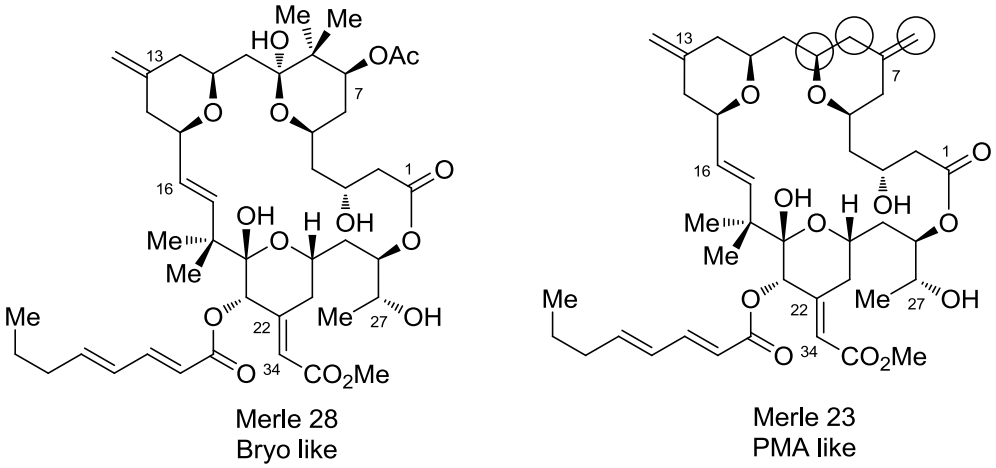


Figure 1.35. Structure of Merle 28 and Merle 23

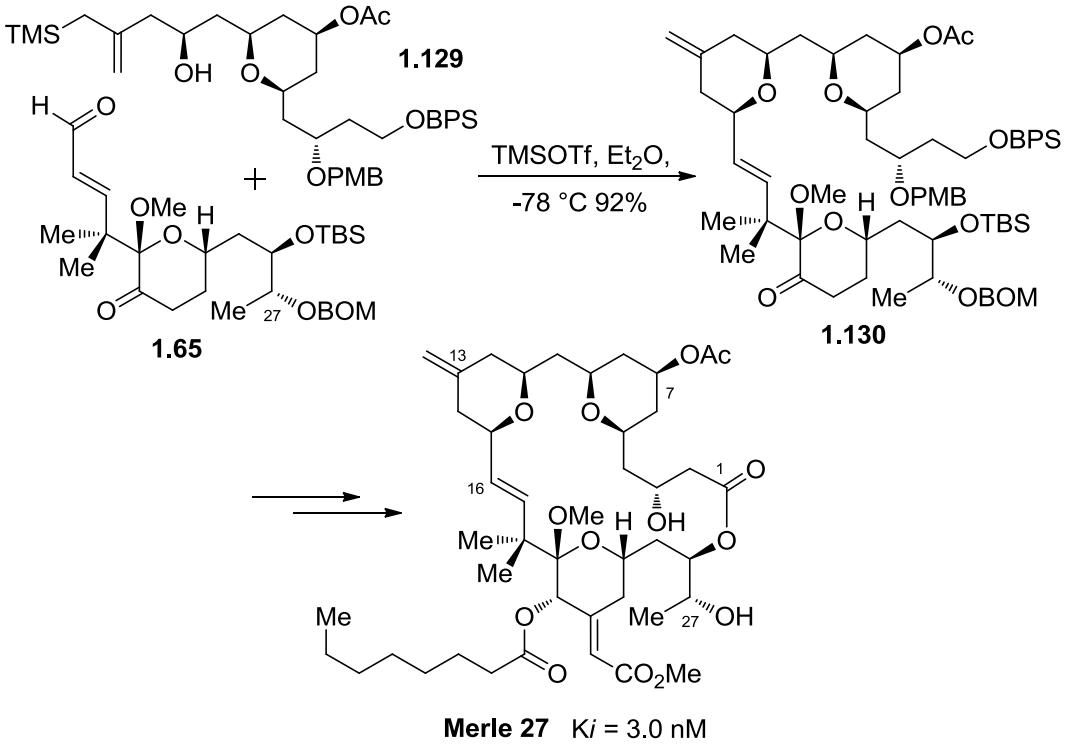


Figure 1.36. Sythesis of C_7-acetate Analogue Merle 27

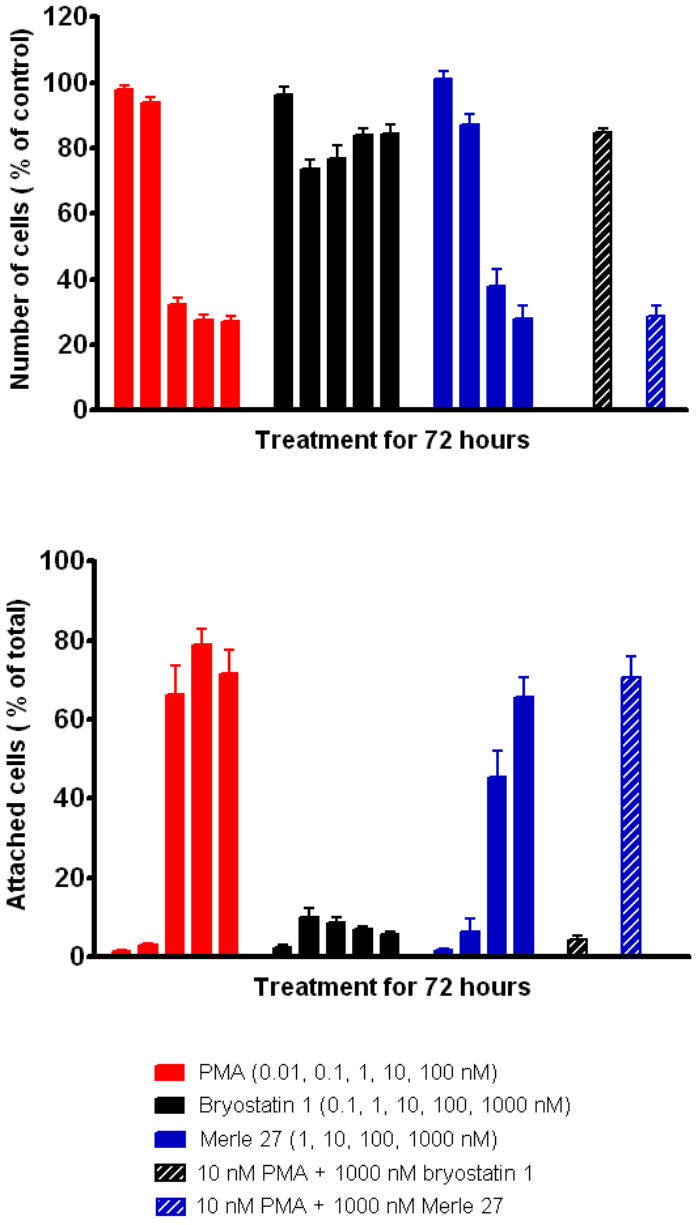


Figure 1.37. Proliferation and Attachment of U937 Cells by Merle 27

of this analogue revealed that it behaved like PMA in attachment and proliferation using U937 cell line indicating that the C_7 acetate is not alone responsible for bryo like biology. Thus the potential positions responsible for PMA/bryo-like behavior of these analogues is finally reduced to two positions, the C_8 gem-dimethyl group and C_9 hydroxyl group.

Synthesis of C_9-Deoxy Bryostatin 1 (Merle 30)

With the information that C_8 and C_9 positions could be responsible for this unusual biological phenomenon, we focussed our attention towards the chemical synthesis and biological study of analogues in which one of these two position were adressed. We thought that the C_9 hydroxyl group could likely be crucial for the PMA/bryo switch in bioactivity. This hydroxyl group had been labile during the synthesis of the Merle 28 even when protected as methylketal group. The unusual reactivity of this group comes from its tendency to form an oxocarbenium ion in acidic medium. The oxocarbenium ion is can be attacked by various nucleophiles including water. We thought a similar phenomenon could be possible in a biological system as well. The oxocarbenium ion being attacked by a nucleophilic protein residue could form a covalent bond between the protein and the A-ring pyran which could lead to bryo-like behavior. Since this functional group is absent in Merle 23 and Merle 27, it seemed to be a possible explanation for the PMA like activity of these analogues.

Computational Study of C_9-Deoxy Bryostatin 1

In addition to the chemical observation, there were some computational studies of bryostatin 1 with the C1 domain of PKC which indicated that C_9 hydroxyl group could

play a critical role in determining the biological activity. One such model is proposed by Itai and coworkers in which they mention a H-bond between the C_9 OH and the carbonyl of methionine 239.[62] In addition, we also conducted the docking studies of bryostatin 1 and C_9 deoxy bryostatin 1 with the C1b domain of PKCα. The computational studies were conducted by Dr. Megan Peach from the NIH. In order to do the docking study, a global energy minimum of bryostatin 1 and its C_9 deoxy analogue was calculated which resulted in a conformation identical to that of the bryostatin 1 crystal structure. The optimized model indicated that an intramolecular H-bonding array similar to one observed in the X-ray structure of bryostatin 1 (Figure **1.38**) was maintained.

In the next step, the lowest energy conformations of both bryostatin 1 and C_9 deoxybryostatin 1 were docked into the protein which revealed that binding of both of these ligands to the C1 domain does not affect their conformations. It was observed from the docking that the A and B-rings of both ligands lie above the binding site in close proximity to the lipid bilayar. In addition to intramolecular H-bonding, both of these ligands make similar H-bonds with PKC residues. Three of these H-bonds are similar in both ligands; (1) a weak H-bond between the C_1 ester oxygen and the NH of Gln 257, (2) a bifurcated H-bonding between the C_{26} hydroxyl and the NH of Thr 242 and carbonyl of Leu 251; and (3) an H-bond between the C_{34} carbonyl and the NH of Gly 253. Similar to the model proposed by Itai, there exists a fourth H-bond between C_9 hydroxyl of bryostatin 1 and the carbonyl of Met 239. This H-bonding is absent in C_9 deoxy bryostatin and more importantly in the phorbol ester compounds suggesting that bryostatin analogues devoid of such H-bondings might behave like PMA.

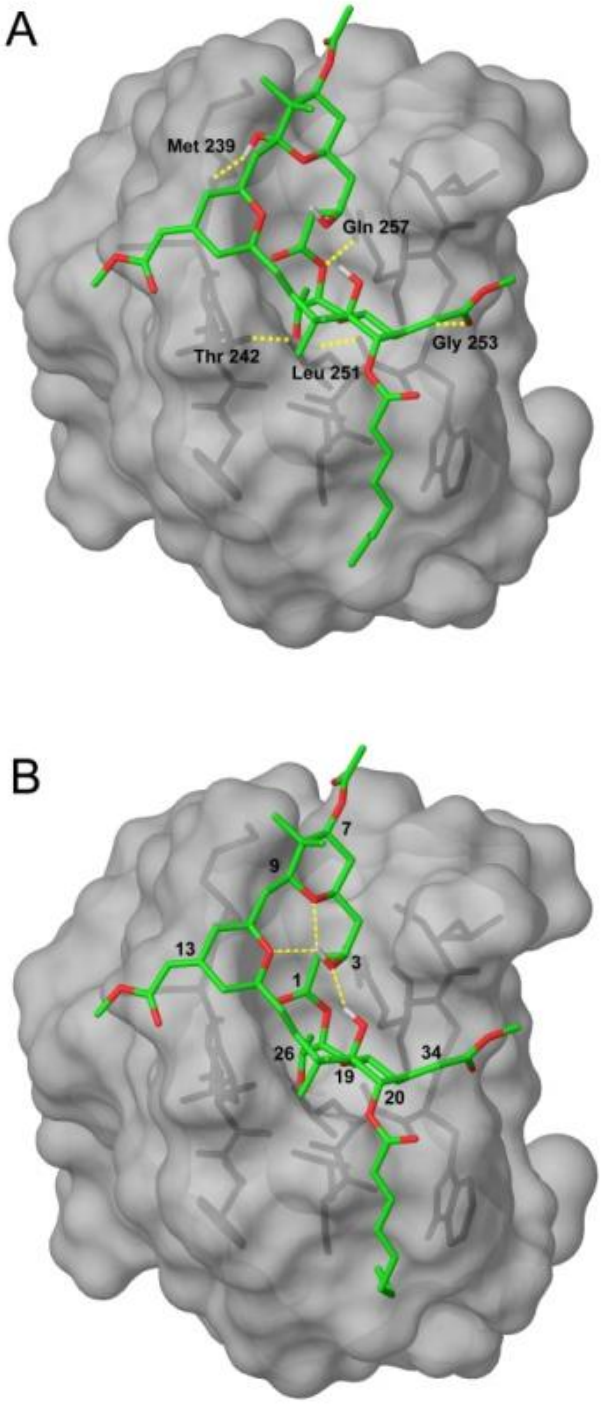
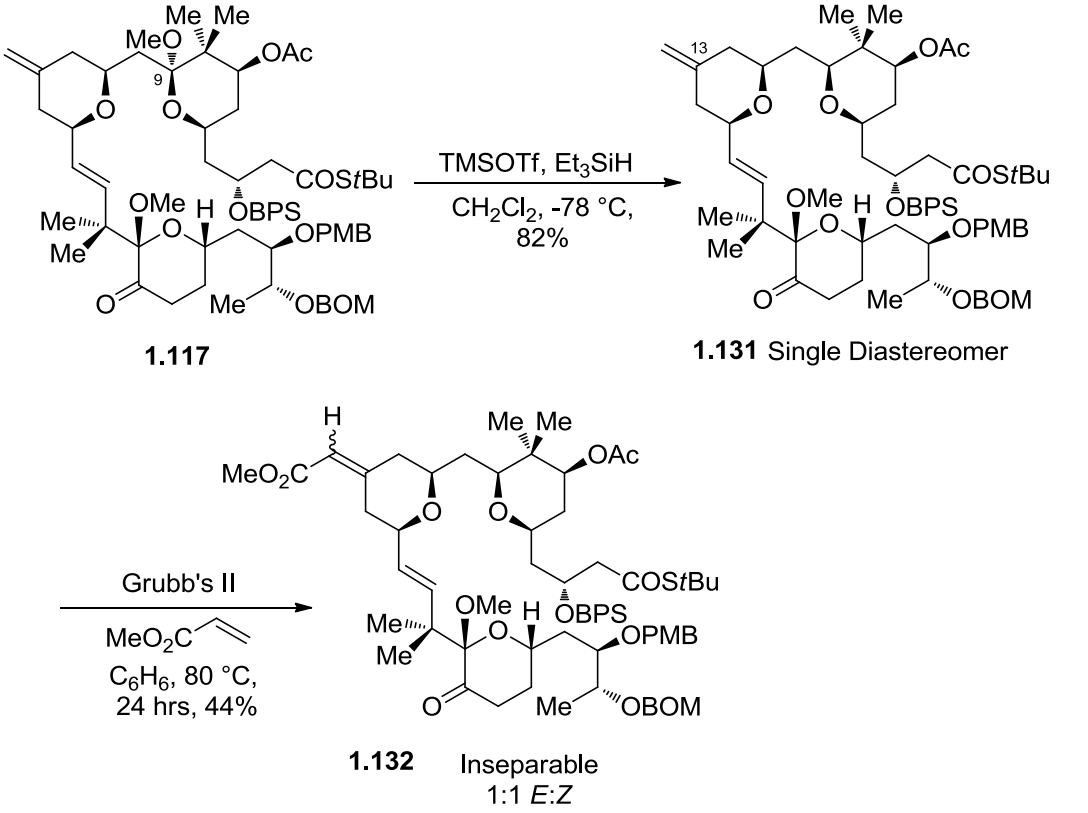


Figure 1.38: Space Filling Model for the Docking of Bryostatin 1 and C_9-Deoxy Bryostatin 1 to PKC C1 Domain

With this information in hand, we planned to check our hypothesis that the C_9 hydroxyl group might act as molecular switch for biological activity. This required the chemical synthesis of C_9 deoxy bryostatin 1 and its biological evaluation. Since the target compound C_9 deoxy bryostatin 1 differs from bryostatin 1 in just one position, we initially thought of doing a relay synthesis using bryostatin 1 as the starting material. This would require a selective deoxygenation of the C_9 hemiketal in the presence of C_{19} hemiketal. Such selective deoxygenation could possibly be carried out since the C_{19} hemiketal is more sterically hindered and electronically deactivated compared to the C_9 hemiketal. The latter is so labile that it was one of the biggest challenges during the synthesis of Merle 28. Moreover, the Masamune group had observed that when the C_{20} ester is acetate, the hydrolysis of the C_{19} methylketal could not be carried out. With this information in hand, we planned to use natural bryostatin 1 as starting material for this synthesis. The synthesis would require several milligrams of bryostatin 1. Unfortunately, the extremely high cost of bryostatin 1 (ca US $2000 per mg), due to its scarcity, prevented us from executing this plan.

The total synthesis of C_9 deoxy bryostatin commenced from the advanced intermediate **1.117** which had been prepared during the total synthesis of Merle 28 (Figure **1.39**). As mentioned earlier, we observed that the C_9 methyl ketal was more reactive than the C_{19} ketal towards acid mediated hydrolysis. The advantage of this difference in reactivity was utilized in the deoxygenation of the former methylketal. Thus when the bisketal **1.117** was treated with TMSOTf and triethyl silane, the reaction provided compound **1.131** as a single regio-and stereo-isomer. The relative stereochemistry of the C_9 position was verified by NOE interaction between the C_9, C_7



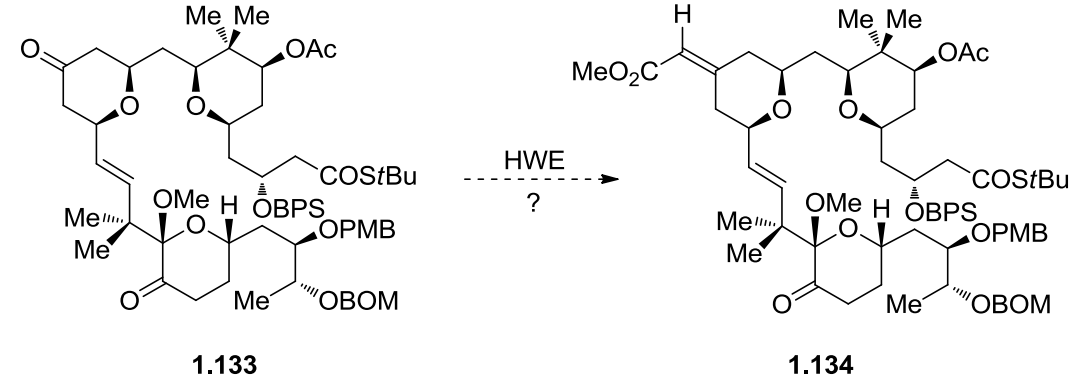


Figure 1.39. Functionalization of A- and B-rings

and C_5 protons. With the successful deoxygenation, the functionalzation of the B-ring was attempted next. Since B-ring already has an olefin handle, a cross metathesis with methyl acrylate was attempted to install the C_{13}-eneoate. The reaction was found completely regioselective towards the exocyclic olefin but unfortunately provided unseparable 1:1 mixture of *E*:*Z* isomers in poor yield. Since the olefin is almost symmetrical and does not have enough steric bias towards metathesis reaction, an improvement to this reaction would be the introduction of external chirality by using asymmetric metathesis catalyst. But such asymmetric metathesis catalysts are still in the process of development and have not been commercialized yet.

Since cross metathesis failed to selectively install the enoate moiety on the B-ring, an alternative solution to this problem was sought. Previous bryostatin syntheses have utilized a modified asymmetric Horner-Wadsworth-Emmons reaction using Fuji's chiral BINOL phosphanate to install this moiety.[27,64] Thus it was thought that we could employ the same reagent to install the ester on the B-ring. This would require a regioselective oxidative cleavage of the B-ring olefin in the presence of the C_{16}-C_{17} olefin. Even though these two olefins are similar electronically, they seem to be in very different steric environment as C_{16}-C_{17} is more sterically hindered environment due to an adjacent gem-dimethyl group.

When the bisolefin **1.117** was treated with OsO_4, we were surprised to find that both of the olefins reacted with the reagent (Figure **1.40**). Attempts to differentiate these two olefins during osmylation by lowering the temperature or carefully monitoring the reaction while using stoichiometric OsO_4 failed. Although the C_{16}-C_{17} olefin seems to be more sterically hindered in flat chemdraw structure, it does not take into account the three

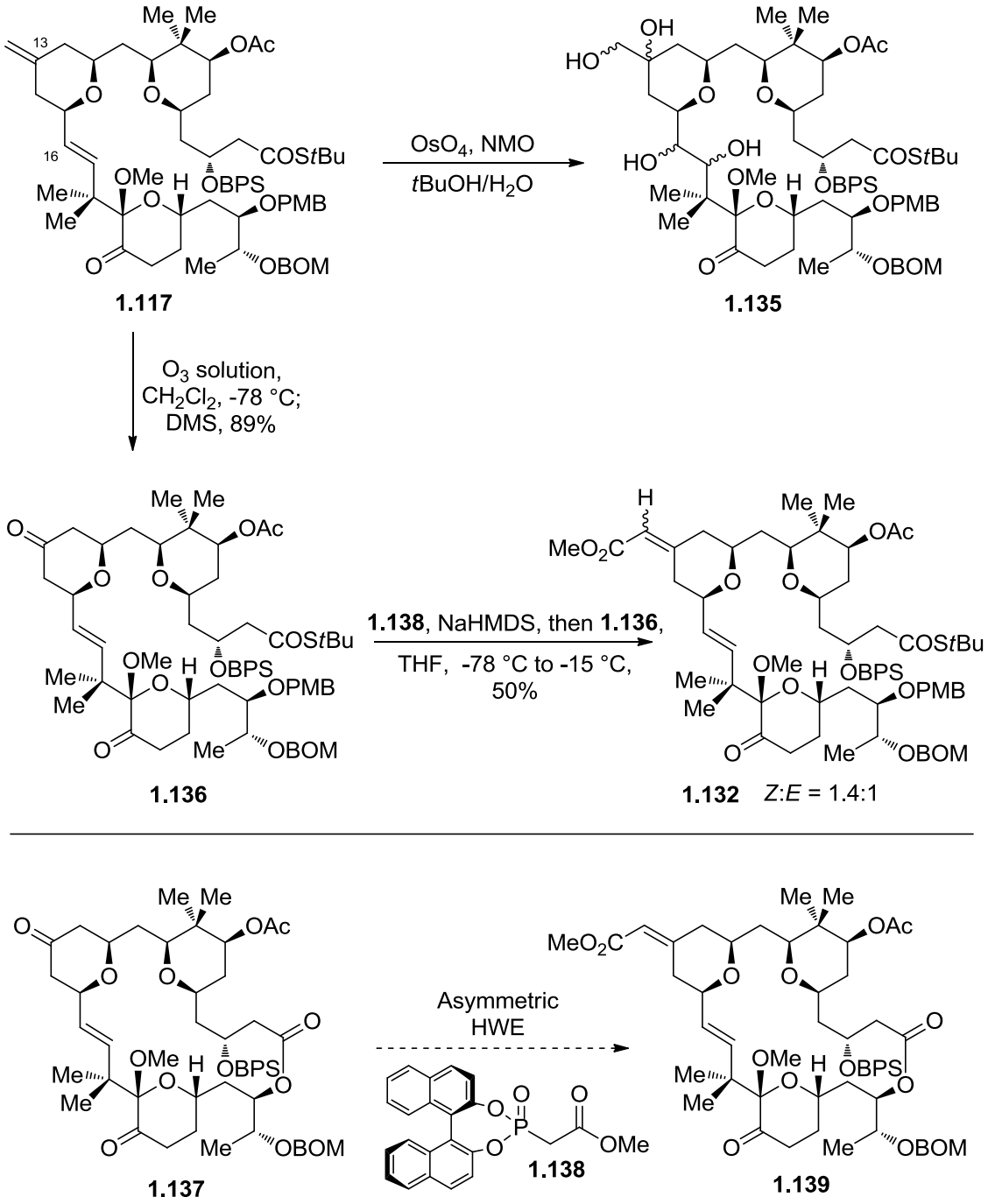


Figure 1.40. Functionalization of B-ring Olefin

dimensional structure of the molecule which could be ultimately responsible for such a low discrepancy during osmylation reaction. With no success in differentiating the olefins, the next reagent to try was ozone. At first, we were little skeptical about using ozone regarding as it is more reactive than osmium tetroxide. Thus theoretically ozone would be less selective for these two olefins if the selectivity depended on the reactivity of the reagent. This was the case when the ozonolysis was done using an ozone stream. But we were delighted to find that when the bisolefin **1.117** was slowly treated with a solution of ozone in CH_2Cl_2, the reaction was found to be regioselective and provided the bisketone **1.136** after reductive workup. It is interesting to note that although both olefins of the substrate **1.117** reacted with OsO_4, when this reaction was later carried out on macrolactone substrate, only the exocyclic olefin reacted with OsO_4. This supports our hypothesis that the two olefins are much more differentiable when they are present in a macrolactone. When bisketone **1.136** was subjected to an asymmetric HWE reaction, the reaction once again provided 1.35:1 mixture of *Z*:*E* olefins which could not be separated. However, the reaction provided only one regioisomer indicating that C_{20} ketone is less reactive towards this reaction presumably due to steric overcrowding and electronic deactivation because of adjacent pyran and methylketal oxygen functionalities.

When similar asymmetric HWE olefination reactions on the B-ring were examined in previous bryostatins syntheses, it was found that the reaction gave the best selectivity in the macrocyclic compounds.[27,28] With this information in hand, the substrate **1.117** was advanced towards the macrocycle (Figure **1.41**). The PMB group was removed under standard oxidative conditions providing the alcohol in excellent yield. Hydrolysis of the thioester was achieved using *m*-CPBA in aqueous THF. We were

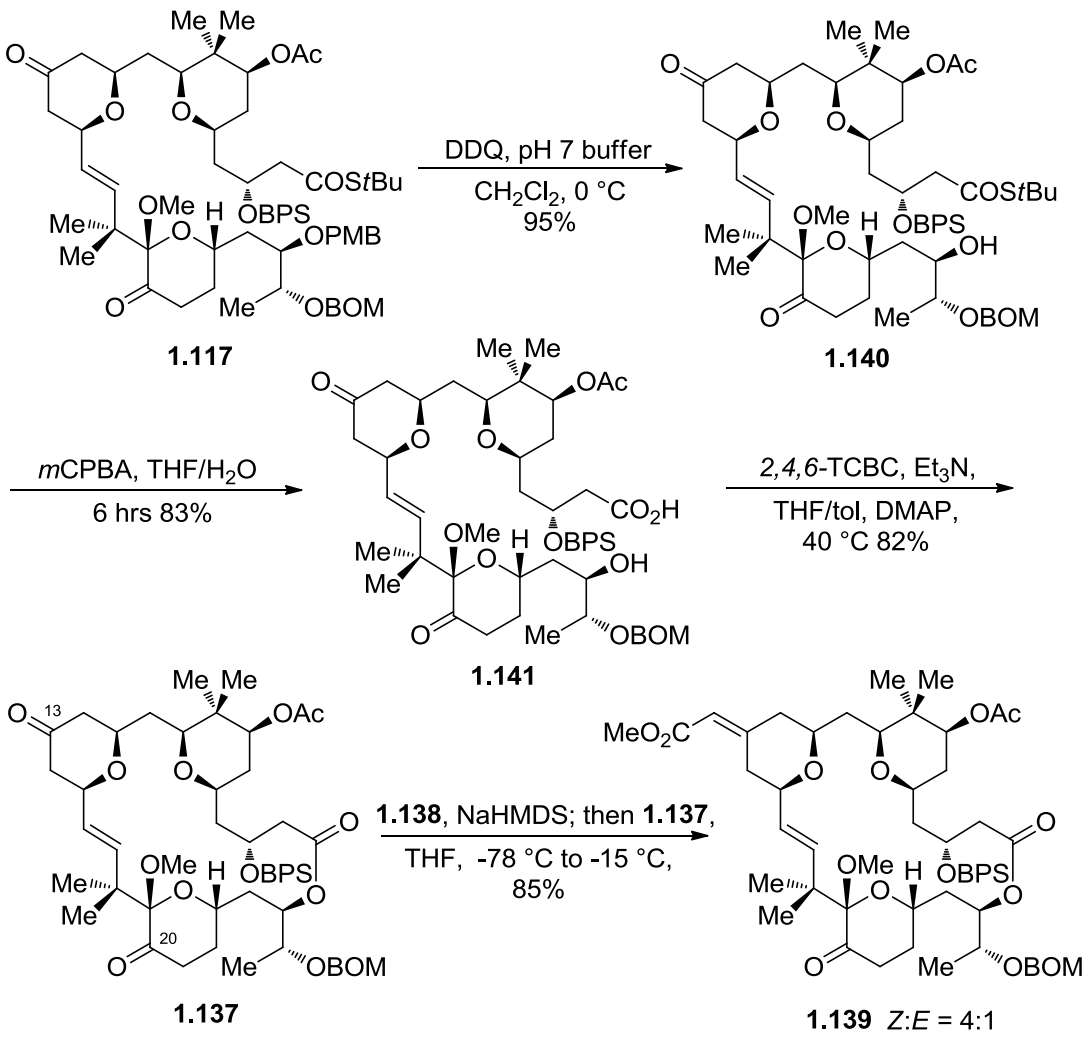


Figure 1.41. Functionalization of the B-ring

pleased to find that that *m*-CPBA did not react with the C_{16}-C_{17} olefin nor did it cause any Baeyer-Villiger oxidation of the ketones on the B and C-rings.[65] Yamaguchi macrolactonization of the seco acid provided the macrolactone **1.137** in good yield. When the bisketone **1.137** was subjected to an asymmetric HWE reaction, the reaction provided the desired compound **1.139** as a single regioisomer and a 4:1 mixture of *Z*:*E* isomer. The desired *Z* isomer could be separated from the mixture using preparative thin layer

chromatography. The expected stereochemistry of the *Z* olefin was verified by observing a NOE interaction between the C_{30} proton and the equatorial proton on C_{12}.

The next step in the synthesis is the functionalization of the C-ring (Figure **1.42**). would cause elimination of the adduct and install α,β-unsaturated ester at C_{21} position resulting a bright yellow enoate. But treatment of the aldol adduct with acetic anhydride resulted in no such product formation. Instead, the reaction led to the formation of a

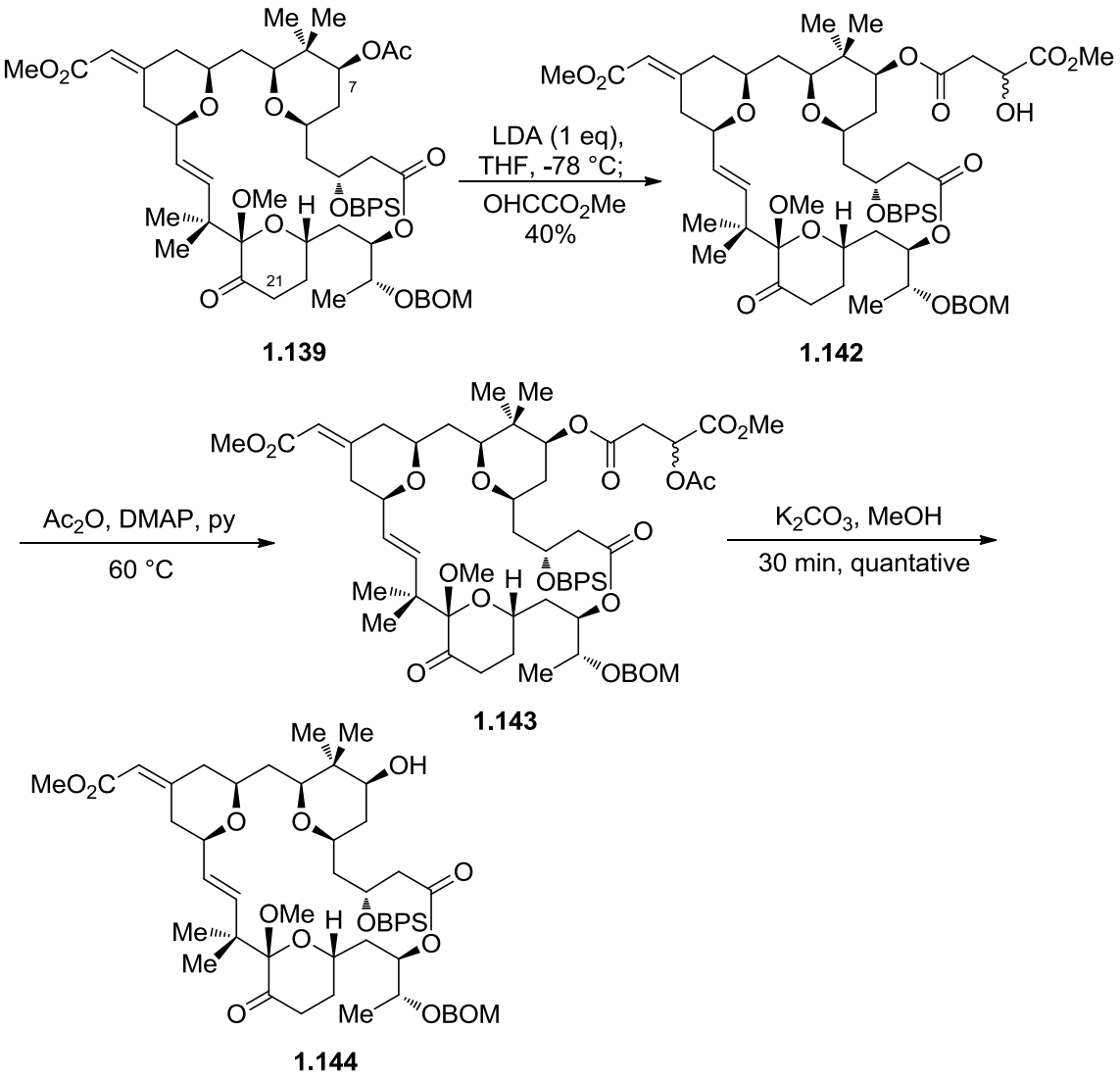


Figure 1.42. Attempted Functionalization of the C-ring

different compound which turned out to be the acetate of the aldol adduct on C_7 acetate. The exclusive formation of such undesired adduct was surprising because the same reaction using one equivalent of LDA provided only the desired product for compound **1.117.** It is very likely that the lack of steric hindrance imposed by methoxy group at C_9 position led the LDA to attack at C_7 position preferentially over the C_{21} position. When the undesired product **1.143** was treated with potassium carbonate in methanol, alcohol **1.144** was generated quantitatively, which could be converted back into the starting material. The stability of the macrolactone ester in this reaction led us to assume that an aldol reaction involving K_2CO_3/MeOH could be used to install the enoate on the C-ring. This reaction, which involves K_2CO_3/MeOH and the methyl acetal of methyl glyoxylate, has been successfully used for a similar reaction in simpler substrates.[32] When ketone **1.139** was stirred with K_2CO_3/MeOH and the methyl acetal of methylglyoxylate, the reaction provided the aldol adduct **1.145** in which the acetate on the C_7 position was removed (Figure **1.43**). Heating of this crude aldol adduct with acetic anhydride and DMAP in pyridine dehydrated the aldol adduct as well as reinstalled the C_7 acetate. The C_{20} stereocenter was installed by reducing the corresponding ketone under Luche conditions providing an unstable alcohol as a 4:1 mixture of diastereomers. The crude alcohol was subjected to esterification with octadieonic anhydride which installed the bryostatin side chain. The undesired minor diastereomer from the Luche reduction was removed at this point using a preparative thin layer chromatography. Removal of the BPS group followed by global deprotection using $LiBF_4$ afforded C_9 deoxy bryostatin 1 or Merle 30.[66]

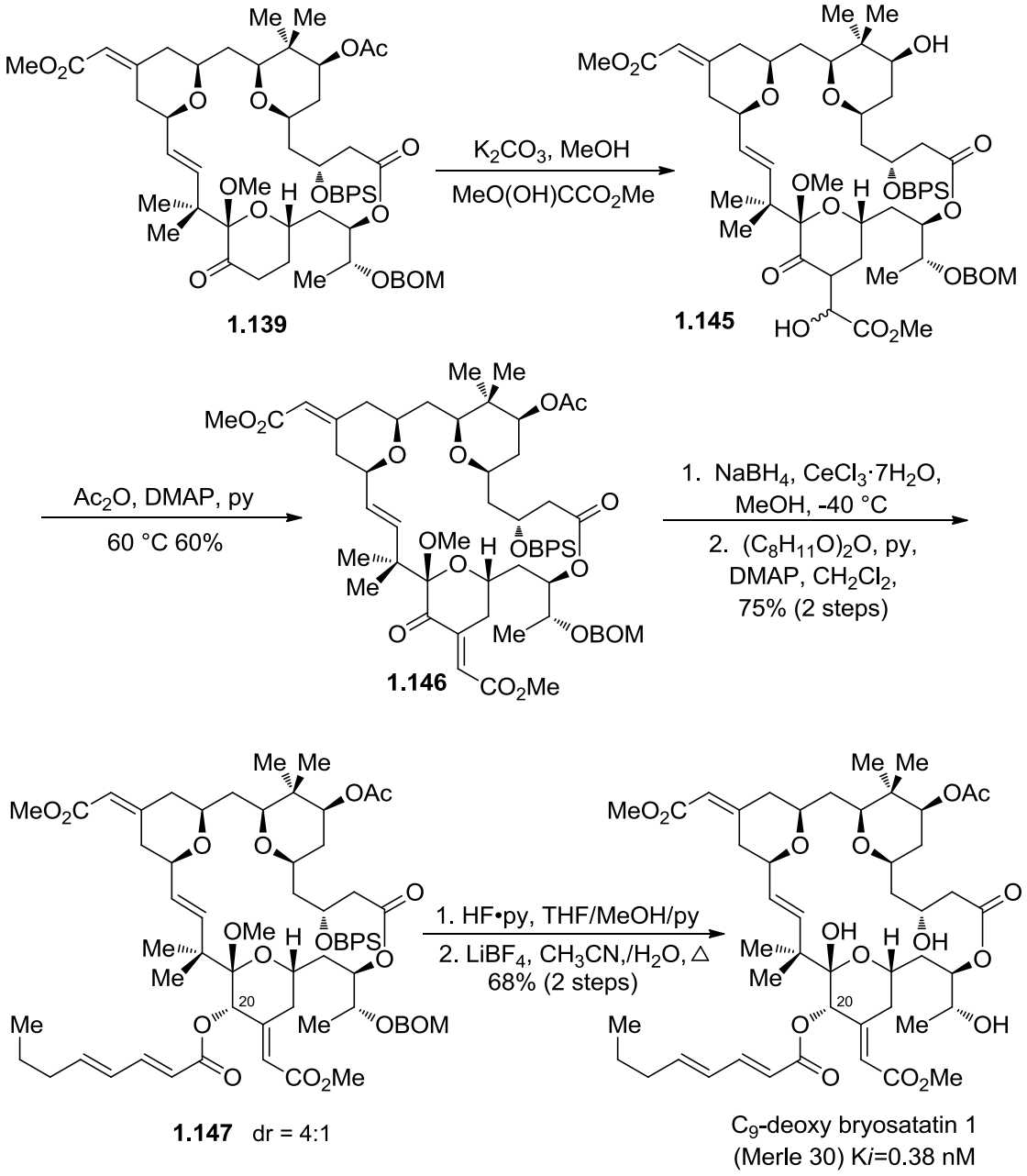


Figure 1.43. Completion of the C_9-Deoxy Bryostatin 1

An interesting observation was made during global deprotection of macrocycle **1.147** (Figure **1.44**). When compound **1.147** was subjected to deprotection using $LiBF_4$, a new spot in the TLC appeared after 1 h which was less polar than starting material. This was surprising since the deprotection of **1.147** would give free alcohols which would make the compound more polar. Observation of the crude NMR of this compound showed all protecting groups were gone except the BOM group. This abnormal behavior of compound **1.148** is believed to be the result of the multiple intramolecular hydrogen bonds in the molecule. It has been observed in the crystal structure of bryostatin 1 that there are two intramolecular H-bonds. The first one is the C_{19}-OH with the C_3-OH and the second one is proton of the C_3-OH making a bifurcated H-bonding with the pyran oxygens of the A and B-rings of bryostatin. Since the BOM-compound is highly analogous to bryostatin 1, similar H-bonding can be expected. Thus the H-bonds tied the free alcohols and resulted in a less polar compound. Once the BOM group was removed,

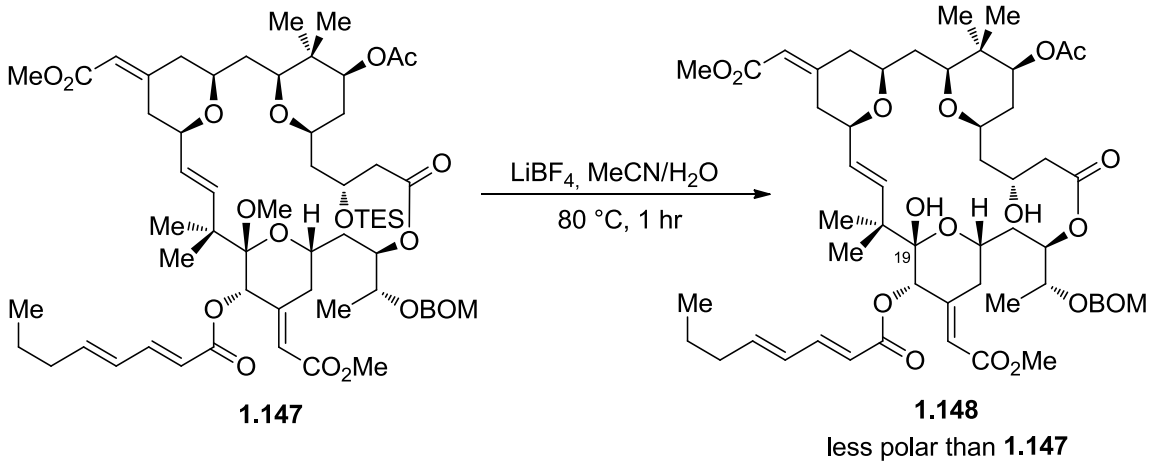


Figure 1.44. Observation of Unexpected Polarity

the resulting final compound was more polar than both the fully protected and the BOM-protected compounds.

Biologial Evaluation of C_9-Deoxy Bryostatin 1

Similar to Merle 28, the biological evaluation of Merle 30 began by evaluating its binding ability with PKCα. From the binding assay, Merle 30 was found to have an inhibitory dissociation constant (K*i* = 0.38 nM) comparable to that of bryostatin 1 (K*i* = 1.35 nM). This suggested that the absence of H-bonding between the C_9-OH and Met 239 of PKCα does not affect its binding ability. According to the computational study and chemical observation, the molecule is held by three intramolecular H-bonds even in the absence of C_9-OH. Thus the presence or absence of the C_9-OH does not change the conformation of Merle 30. Since bryostatin is extensively bound to the C1 domain of PKCα which involves four H-bonding interactions and various other nonspecific interactions, the loss of binding due to the C_9-OH hydrogen bond might have been counterbalanced by an increase in nonbonding interaction due to the increase in the hydrophobicity of the A, B-ring region.

The second part of the biological investigation involved the determination of the function of Merle 30 with respect to PMA. This was again carried by proliferation and attachment cell assays using the U937 cell line. It appears from the attachment assay that Merle 30 induced cell attachment similar to bryostatin 1 but to a slightly larger extent (Figure **1.45**). Similar to Bryostatin 1, Merle 30 exhibited the biphasic behavior and blocked the effect of PMA in dose dependent manner. In addition, it was shown to behave similar to Merle 28.

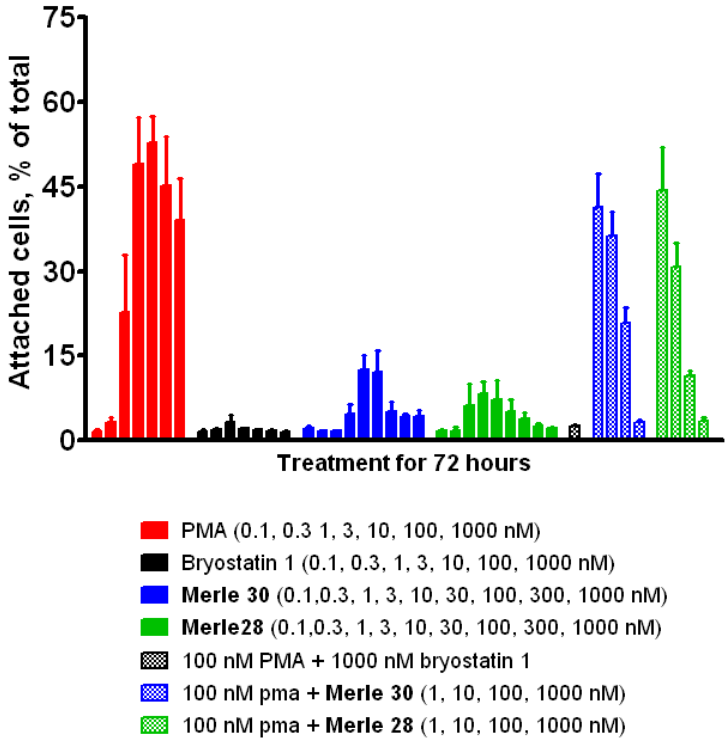


Figure 1.45. Attachment of U937 Cells by Merle 30

In the second assay, the proliferation caused by Merle 30 is very different from that caused by PMA and very similar to bryostatin 1 (Figure **1.46**). In both of the assays, Merle 30 blocked the effect of PMA in dose dependent manner. Thus these assays suggested that Merle 30 functions like bryostatin and not like PMA. In order to determine whether the functional behavior exhibited by these analogues is general or not, another assay was carried out using LNCaP cell line. These are androgen-sensitive human prostate cancer cell lines widely used in various onological studies.[67] Similar to the U937 cell line, these cells also give different responses to PMA and bryostatin, PMA is strongly antiproliferative while bryostatin 1 is not. Moreover, bryostatin 1 blocks the effects of

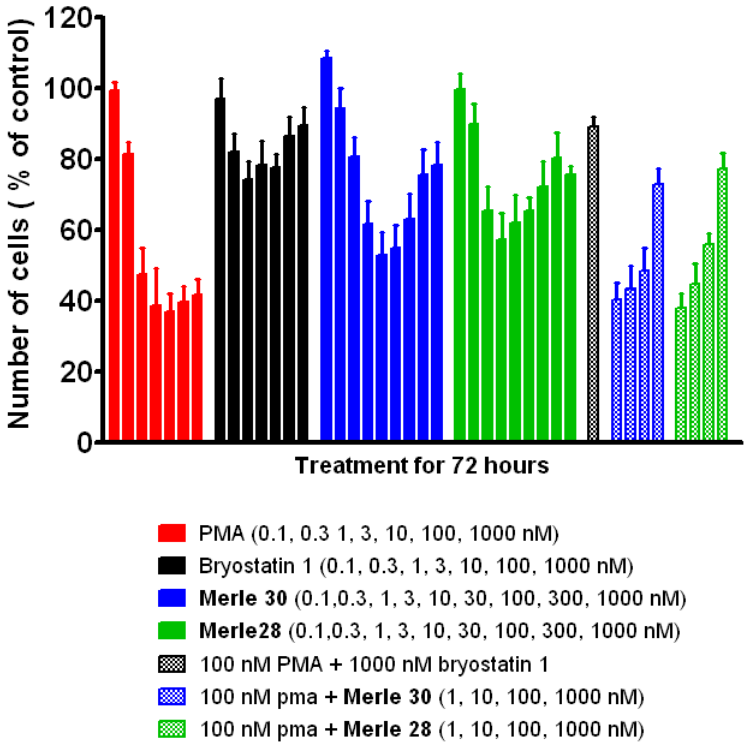


Figure 1.46. Proliferation of U937 Cells by Merle 30

PMA which it itself does not exhibit.

As seen from the proliferation assay using LNCaP cells (Figure **1.47**), Merle 30 shows response similar to that of bryostatin and different from that of PMA. Moreover it also blocks the effect of PMA in dose dependent manner, a characteristic behavior of bryostatin 1. Another assay involving LNCaP cell is the effect of these ligands in the secretion of tumor necrosis factor α (TNF–α). TNF–α is a immunomodulating protein produced mostly by immune cells and induces inflammatory response. Phorbol esters such as PMA induce the production of TNF–α whereas bryostatin has little effect and it also blocks the effect of PMA when administered together. According to this assay, PMA

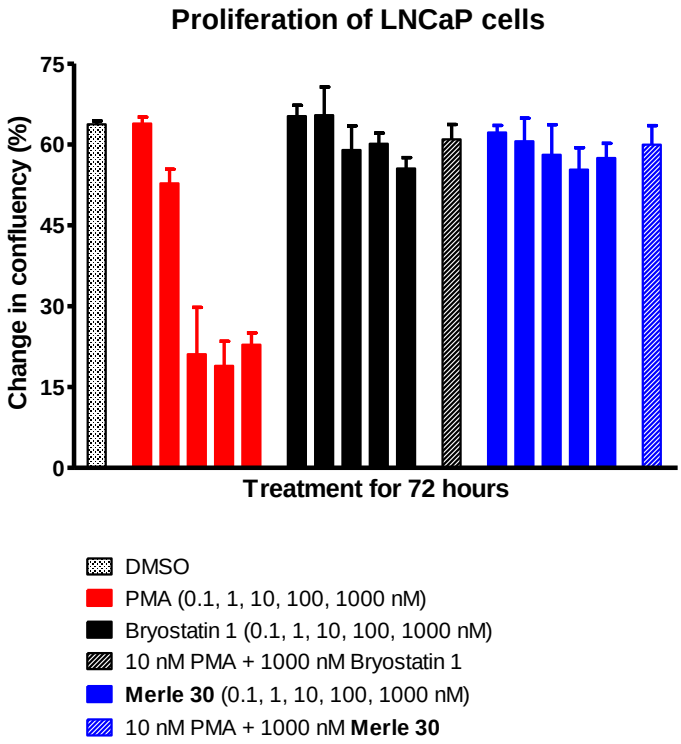


Figure 1.47. Proliferation of LNCaP Cells by Merle 30

induces the highest secretion of TNF–α in a biphasic manner whereas bryostatin 1 has little effect. Moreover bryo blocks the effect of PMA in a dose dependent manner. It is seen from the assay that Merle 30 has little effect on the secretion of TNF–α and more importantly blocks the secretion caused by PMA when both agents are applied together (Figure **1.48**). This clearly suggests the bryostatin like nature of Merle 30. Even though Merle 30 is very similar to bryostatin 1 in both of these assays; it also has a small PMA like character shown by small increase in attachment of the U937 cells, decrease in the proliferation of both U937 and LNCaP cells as well as small increase in the secretion of TNF–α.

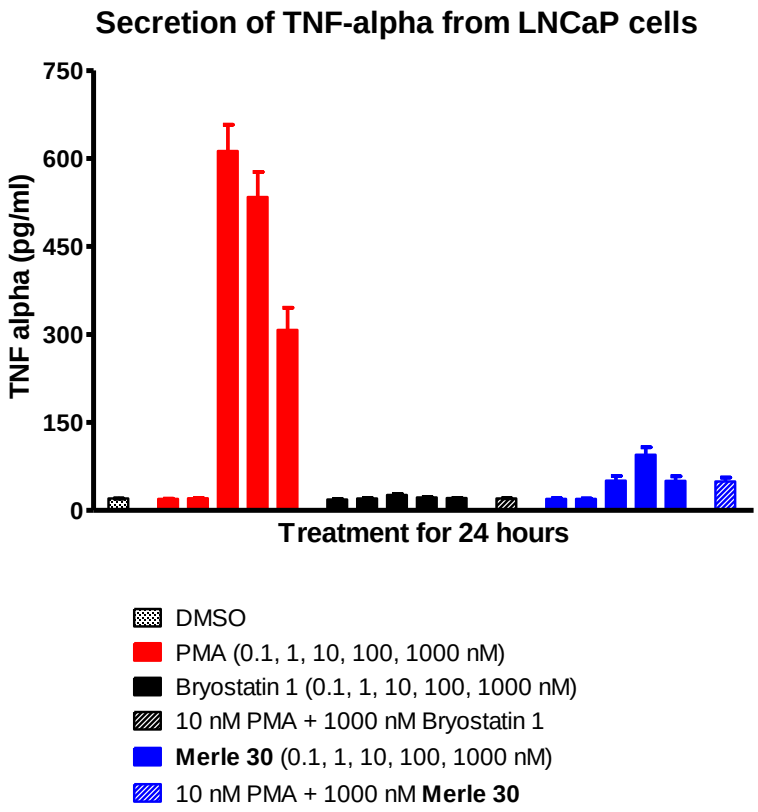


Figure 1.48. Secretion of TNF-α from LNCaP Cells by Merle 30

From the study of the biological activity of various bryostatin analogues it was assumed that two groups on the A-ring could be responsible for the bryostatin like activity exhibited by these analogues, the C_8 gem-dimethyl group and the C_9 hydroxyl group (Figure **1.49**). The C_9 hydroxyl group was thought to contribute more in the biological response both due to its chemical reactivity and ability to H-bond with the C1 domain of PKC. It is clear from the study of Merle 30 that the absence of C_9 hydroxyl group has very little affect in its binding ability to PKC–α. Moreover, the biological through the function of the Merle 30 is not much affected by the absence of C_9 hydroxyl

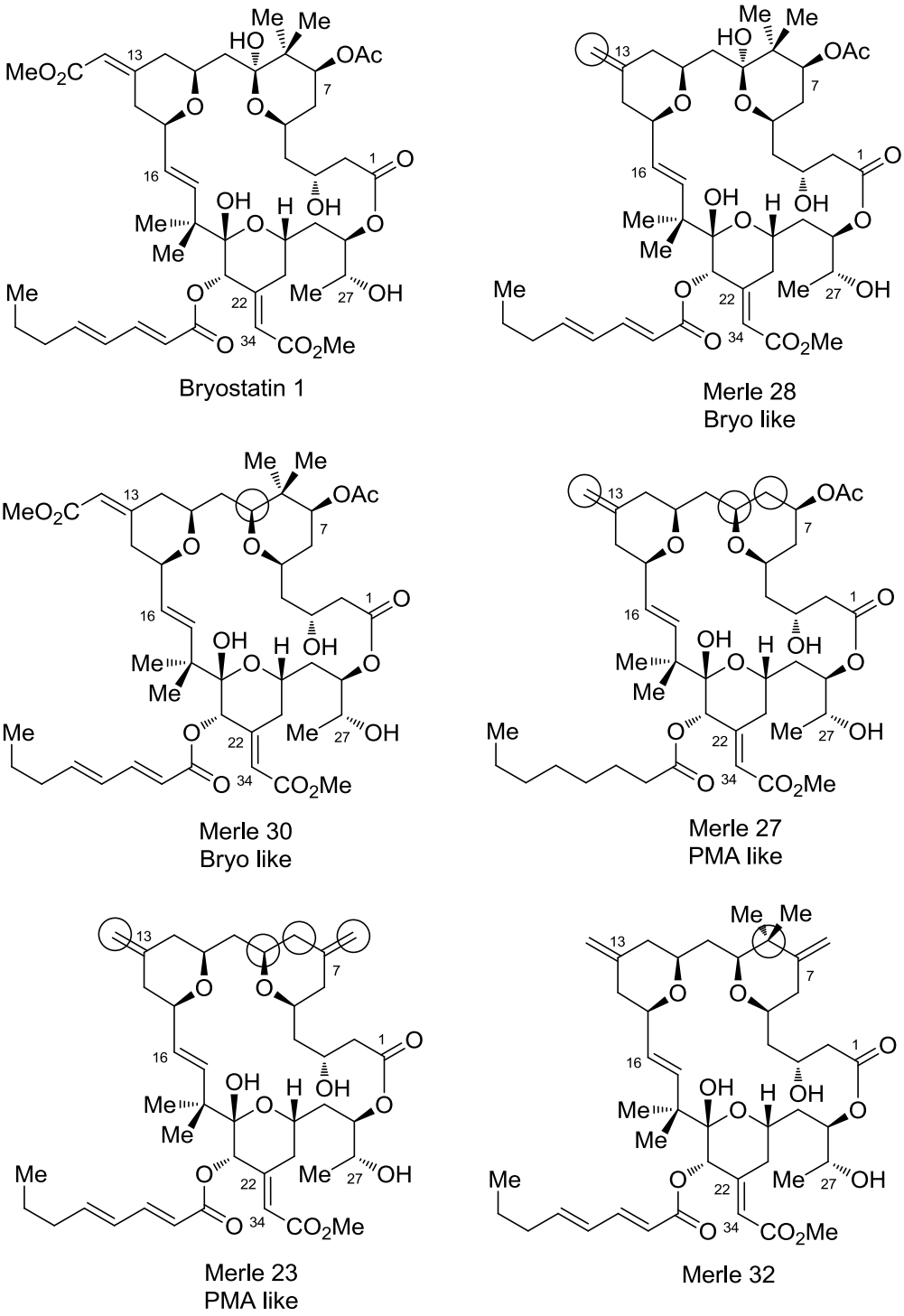


Figure 1.49. Difference Among Various Merle Analogues

group. Thus by chemical synthesis and biological evaluation of various analogues, we have determined the role of various functional groups. Firstly, the biological data from Merle 23 showed that A and B-rings are responsible for the bryostatin like biology. Merle 28 revealed that the ester functional group on the B-ring was not important indicating that substitution on the A-ring was critical. Among the three groups on the A-ring; C_7 acetate, C_8 gem-dimethyl group and C_9 hydroxyl group, the biological results from Merle 27 suggested that C_7 acetate was not critical. This left gem-dimethyl group on C_8 and and C_9 hydroxyl group. From the study of Merle 30, it was revealed that C_9 was not the molecular switch of bryostatin-phorbol biology. This leaves the gem-dimethyl group which could be potentially responsible for the bryostatin like response of these analogues. It is interesting to note that of the all analogous prepared and tested for biological activity from our group so far, those devoid of the gem-dimethyl group behave like phorbol esters whereas those with the gem-dimethyl group behave like bryostatin 1.

It has been observed from the study of the C1 domain of PKC that its top face interacts with the lipid bilayer.[15] When the C1 domain binds with the ligands such as bryostatin and phorbol ester, it changes the surface characteristics of the top face and thus leads to a change in its interactions with the lipid bilayer. Our modeling of bryostatin 1 with the C1 domain of PKC also suggests that binding of bryostatin 1 significantly changes the polarity of lipid binding surface, and thus might result in a change in its interactions with the lipid bilayer. Among the four groups in the A and B-ring of bryostatin 1, which are absent in phorbol like analogue Merle 23, carbomethoxy group, C_9 alcohol and C_7 acetate are oxygenated and more polar. The C_8 gem-dimethyl group is hydrophobic, and one of the unique structural features of bryostatins. Since binding of

bryostatin 1 with the C1 domain changes its interaction with the lipid bilayer, the presence or absence of polar/nonpolar group on bryostatin would make a significant contribution towards this interaction. Such change in the polarity of the ligand could ultimately be responsible for the switch in the phorbol-bryostatin like biological activity of the analogues of bryostatin.

Synthesis of C_8-Gem-dimethyl Analogue (Merle 32)

From the observation of the biological profile of analogues so far studied, it seemed logical that the gem-dimethyl group could be critical in defining the biological activity. The effect of this group could be tested by synthesizing the analogue with only the gem-dimethyl group on the A-ring or one with all the substituent but the gem-dimethyl group on the A-ring. The former would serve as positive control whereas the latter would serve as a negative control. Moreover, the ideal chemical structure would be one which could be compared directly with the closest phorbol-like bryostatin analogue and is preferably different from it only at the gem-dimethyl group. Such an analogue would be Merle 32 (Figure **1.49**) which has a gem-dimethyl group at the C_8 position and differs only at one position from the previously studied phorbol like analogue Merle 23.

Synthetically, it seemed more reasonable to use the existing intermediate and route to synthesize this analogue. This would avoid the time and cost involved in developing a separate route to synthesize and test this analogue. The synthesis commenced from compound **1.131** which was synthesized enroute to C_9-deoxy bryostatin 1. The synthesis would follow essentially the same route as that of Merle 30 with the substitution of the acetate on C_7 position with an exo-methylene group. We envisioned

that hydrolysis of the C_7 ester would reveal an alcohol which could be oxidized to a ketone. The ketone could then be converted to olefin using an olefination reaction. Among all the reactions in the sequence, we were most concerned about the crucial olefination reaction. This is because the ketone is adjacent to a sterically demanding gem-dimethyl group. Proximity of such a severely bulky group would make the approach of the reagent very difficult and lead to side reactions in a complex substrate. With this potential problem in mind, we first decided to test the viability of the olefination reaction on a model substrate. The synthesis of model substrate **1.151** commenced from the previously synthesized intermediate **1.149** (Figure **1.50**). Deoxygenation of the methylketal **1.149** at the C_9 position followed by TBS protection of the C_{11} alcohol gave

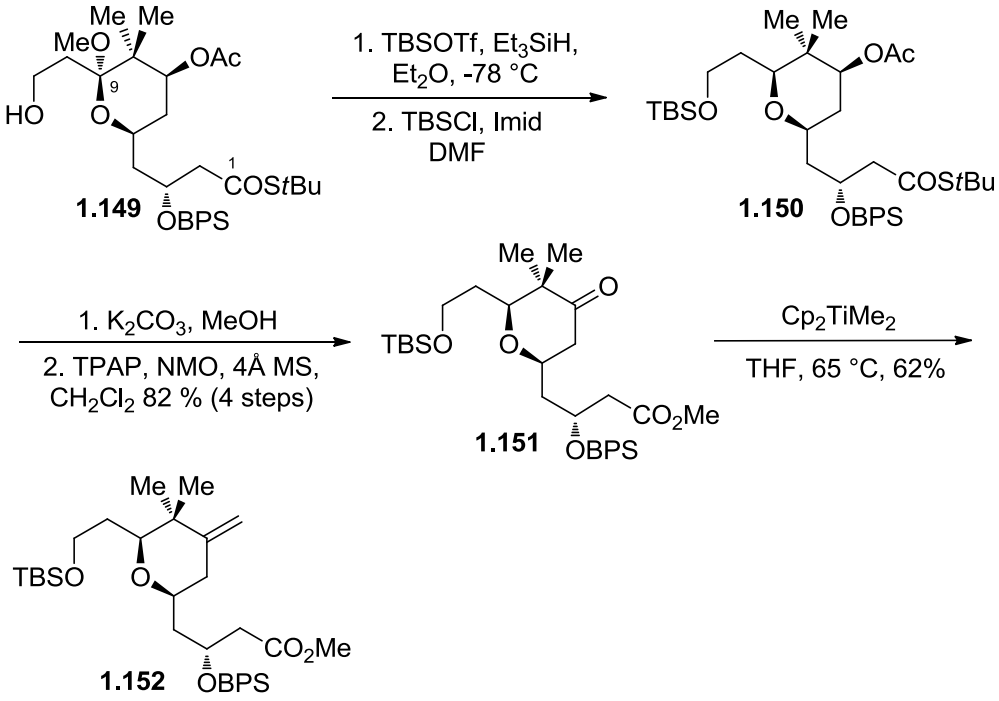


Figure 1.50. Model Olefination Study

compound **1.150**. A transesterification using K_2CO_3/MeOH removed the C_7 acetate and converted the C_1 thioester into a methyl oxoester. The free alcohol was oxidized to a ketone using TPAP/NMP providing the model substrate **1.151** in excellent yield over 4 steps. With **1.151** in hand, we decided to first investigate the use of the Petasis reagent in the olefination reaction.[68] Although the Petasis reagent is also known to react with ester carbonyls, the reactivity can usually be controlled by increasing/decreasing the temperature of reaction. The choice of the Petasis reagent as opposed to the Wittig reagent was made based on the previous observation that C_1 ester compounds with a BPS group on the C_3 position were prone to β-elimination in Wittig reaction conditions.[69] Moreover, a ketone such as **1.151** is in an extremely hindered environment and the Wittig reagent is known to be sensitive towards steric factors. An alternative but similar to Petasis reagent would be Tebbe's reagent.[70] Here we were concerned about the lower reactivity and higher Lewis acidity of the reagent, especially in the context of a complex intermediate. In the event, when ketone **1.151** was subjected to Petasis olefination conditions, the reaction provided the olefin **1.152** in good yield.

With the success of the model olefination reaction, the synthesis of Merle 32 commenced from the previously synthesized intermediate **1.131** (Figure **1.51**). The PMB group was removed under standard conditions providing alcohol **1.153** in excellent yield. Hydrolysis of the thioester was achieved using *m*CPBA in THF/H_2O. During this hydrolysis, the thioester was stirred with *m*CPBA in THF/H_2O at 0 °C for 1 h, and then at room temperature for 2.5 h. It was necessary to stop the reaction after 3.5 h in order to prevent the epoxidation of the B-ring olefin. Although both the product and the byproduct from the reagent are carboxylic acids, the *m*-chlorobenzoic acid could be easily removed

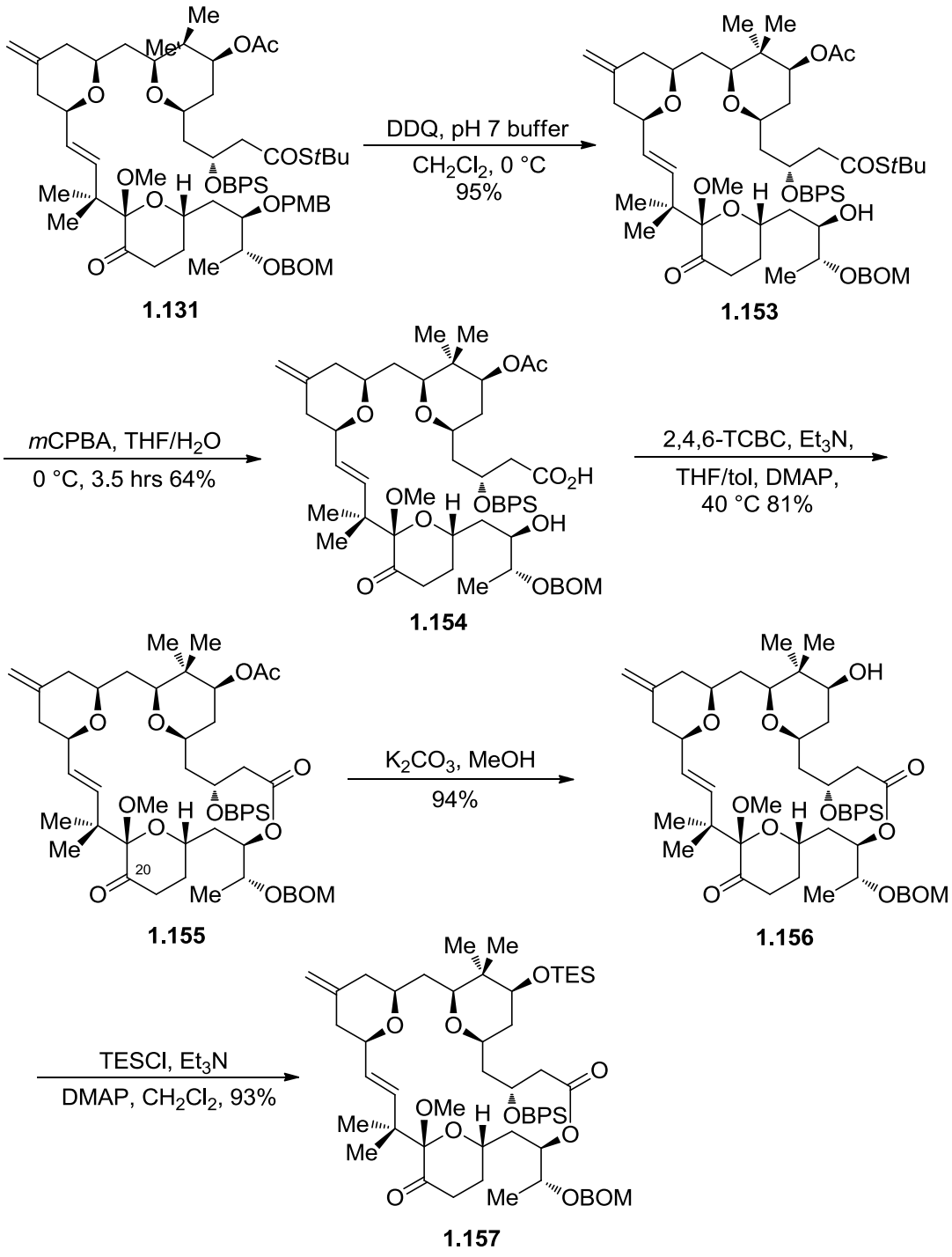


Figure 1.51. Synthesis of Macrolactone **1.51**

from the mixture by silica gel column chromatography using 30% ethyl acetate in hexanes. With the seco acid in hand, a Yamaguchi reaction provided the macrolactone **1.157** in good yield.

The next step in the synthesis would involve the functionalization of the C-ring through an aldol reaction reaction with methyl glyoxylate. Experience from previous analogue syntheses had shown that the aldol reaction could not be done on the C-ring with the C_7 acetate still present in the molecule. Aldol condensation using the K_2CO_3/MeOH was another alternative but that would remove the C_7 acetate. Therefore a cleaner route was devised in which the C_7 acetate was removed using K_2CO_3/MeOH and the resulting alcohol was protected as TES ether (Figure **1.52**). An aldol reaction of the ketone **1.157** using LDA and freshly prepared methyl glyoxylate provided the aldol adduct **1.158** as a mixture of diastereomers. Elimination of the crude aldol adduct to the desired α-keto α,β-unsaturated ester **1.159** was accomplished by stirring the adduct with acetic anhydride and DMAP. It was necessary to keep the elimination reaction at room temperature to avoid the deprotection of the TES group. The C_{20} stereocenter was set by Luche reduction of ketone **1.159,** and the alcohol was immediately esterified with *2*,4-octadieonic anhydride. Removal of the TES group under standard conditions followed by Dess-Martin oxidation of the resulting alcohol provided the ketone **1.161** in excellent yield.

When the ketone **1.161** was subjected to the Petasis olefination reaction, no desired product was formed (Figure **1.53**). Instead, the reaction provided a complex mixture of byproducts which could not be isolated and identified. The failure of this olefination reaction led us to examine other olefination reactions. Although we were

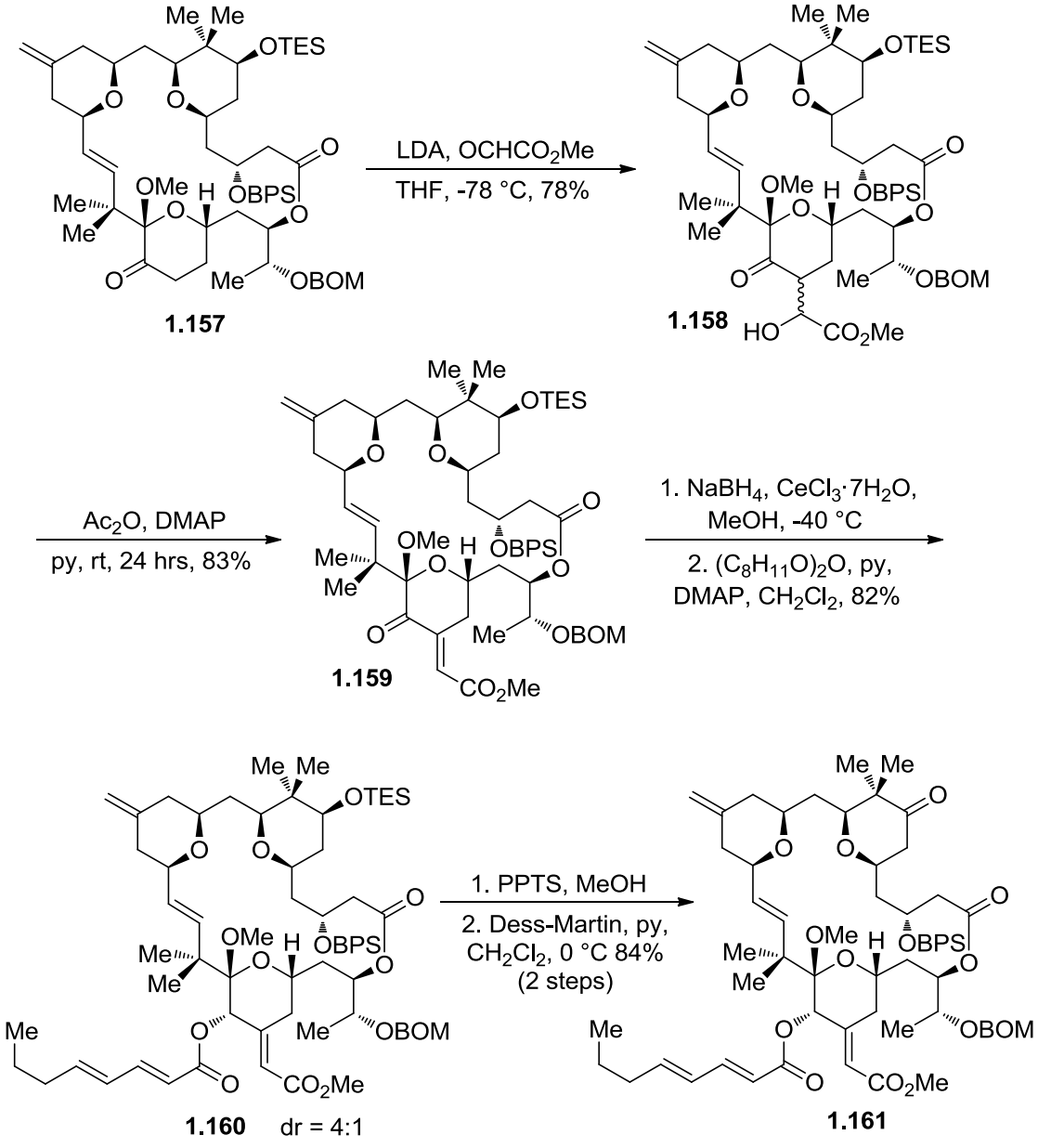


Figure 1.52. Preparation of Ketone **1.161**

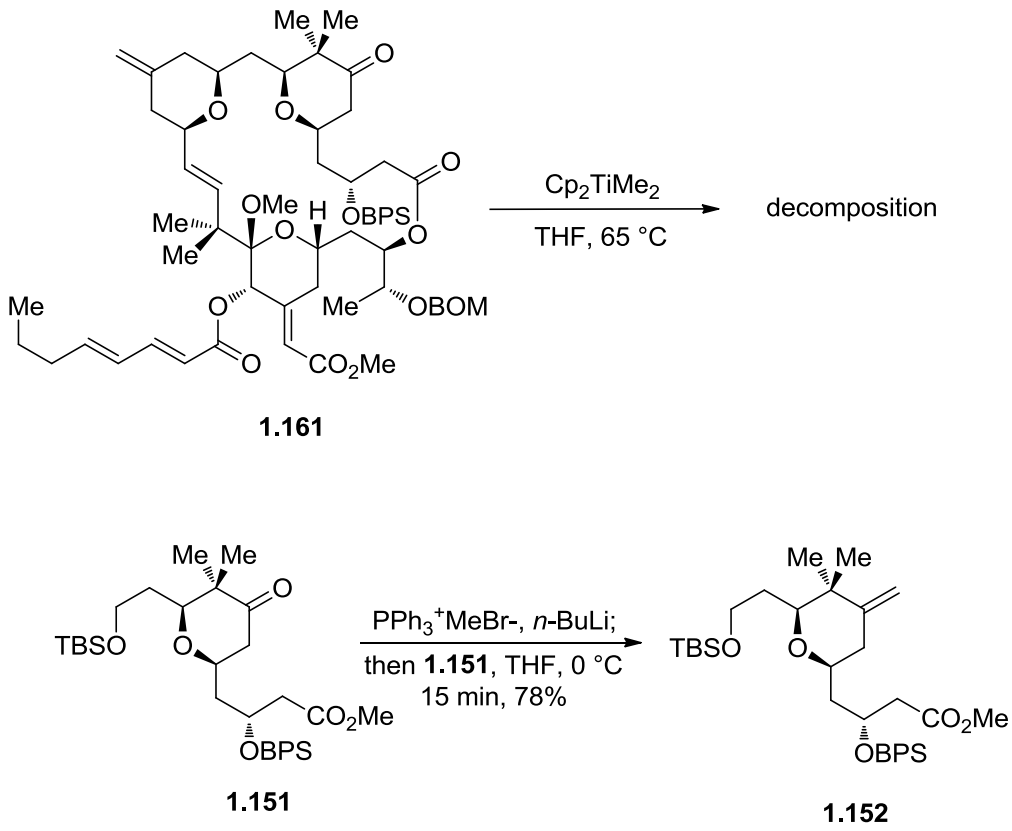


Figure 1.53. Olefination Study

concerned about β-elimination of the model ketone **1.151** under basic condition, we were prompted to try a Wittig reaction on it. We were pleased to find that the Wittig olefination on the model ketone **1.151** actually worked. It was proved to be extremely important to stop the reaction at 15 min in order to avoid decomposition of the desired product and other side reactions. When the reaction was stopped at 15 min, a 78% isolated yield of the desired alkene was realized. When this olefination condition was applied to the advanced substrate **1.161**, the reaction provided the desired olefin **1.162** (Figure **1.54**). Removal of BPS ether with HF•py followed by global deprotection then provided the analogue Merle 32 in good yield.

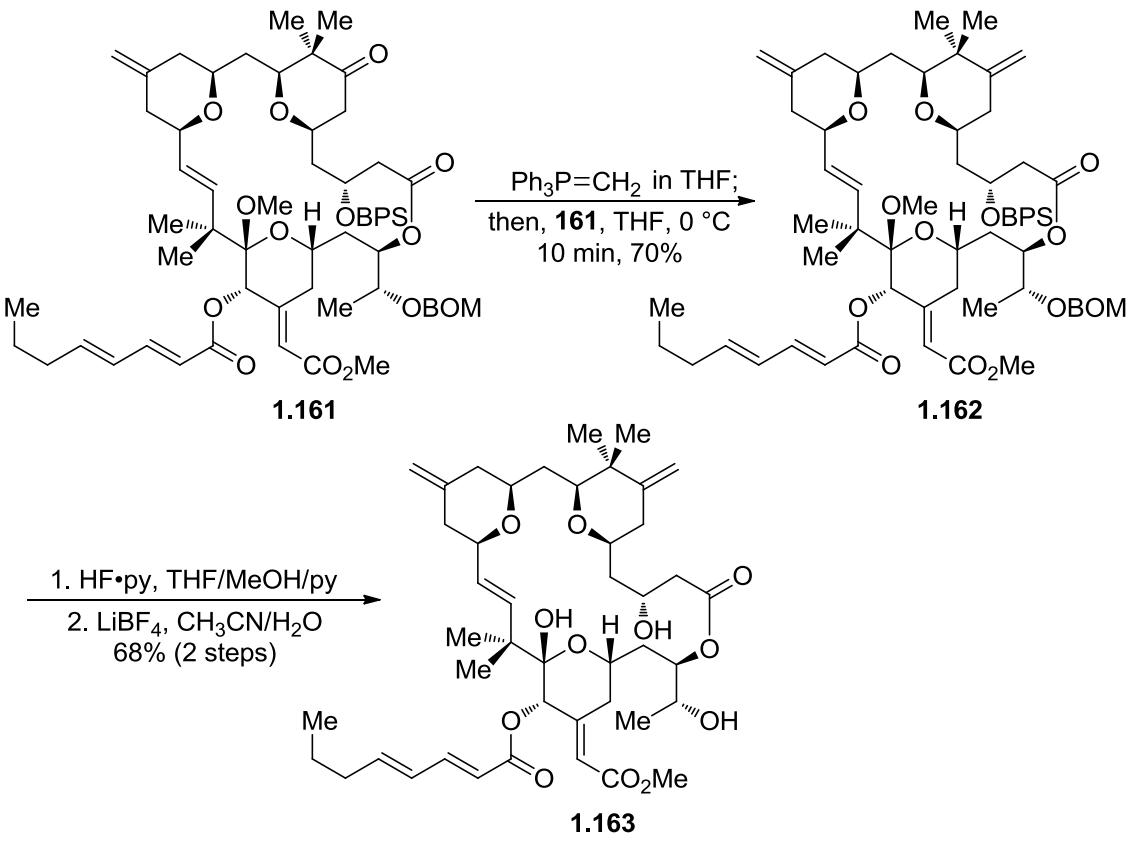


Figure 1.54. Completion of Merle 32

Biological Evaluation of C_8-Gem-dimethyl Analogue

Merle 32 was submitted for the biological evaluation involving its binding affinity to PKC-α and function in terms of bryostatin-phorbol like activity. From the binding assay, the inhibitory dissociation constant (K*i*) was found to be 1.08 nM. This suggested that the presence of the gem-dimethyl group does not affect the binding affinity towards PKC-α.

Further biological activity in terms of function was carried out by proliferation and attachment assays in U937 cell lines (Figure **1.55**) . The proliferation assay involving the U937 cell line revealed that Merle 32 gave biological results very similar to those

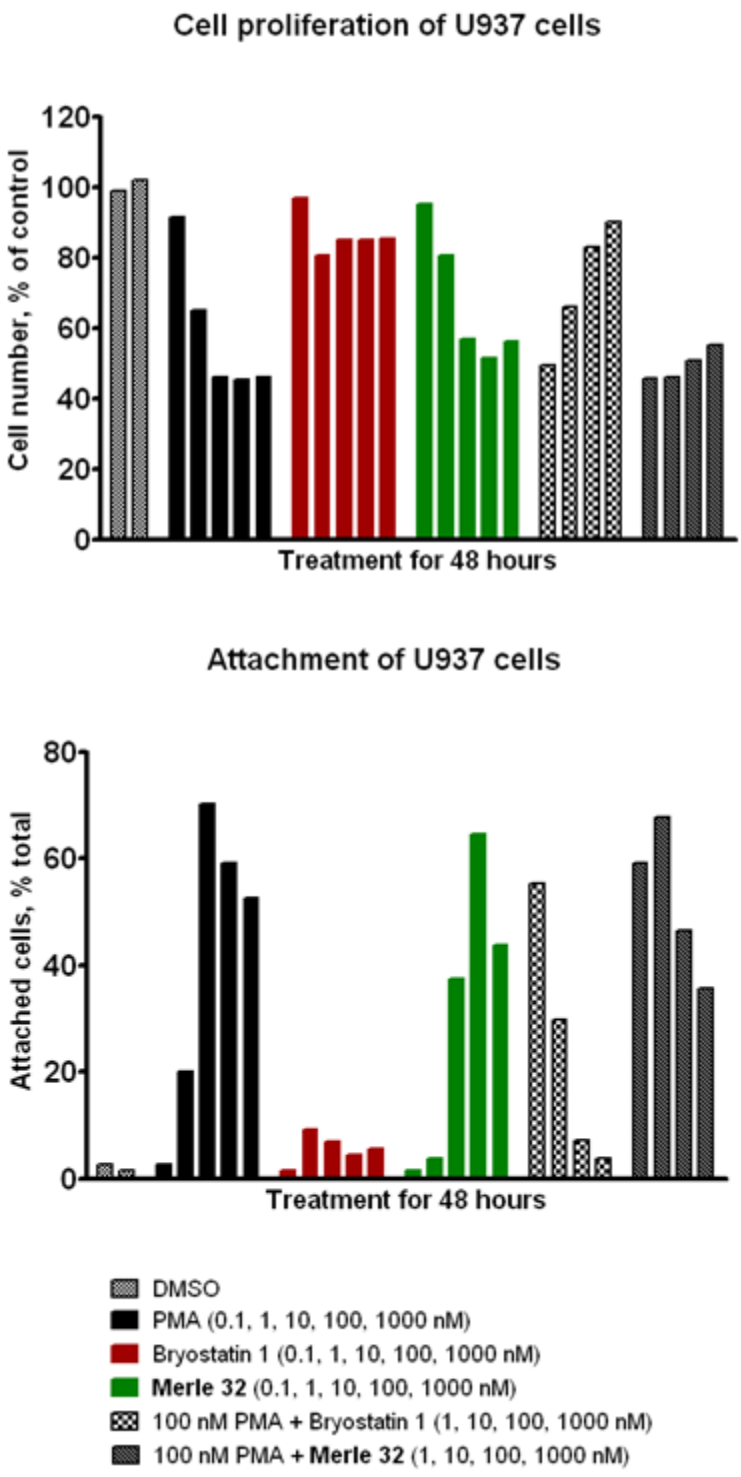


Figure 1.55. Proliferation (top) and Attachment (bottom) of U937 Cells

seen with PMA and much different from bryostatin. Moreover, unlike the previous bryostatin 1 like analogues, Merle 32 was not able to block the effect of PMA when it was administered together with bryostatin 1. In the attachment assay, Merle 32 induced attachment similar to that of PMA and was not able to block the effect of PMA. Both of these assays involving the U937 cell line showed that Merle 32 is a functional antagonist of bryostatin 1.

In order to verify that the PMA like behavior displayed by Merle 32 in the U937 cell line was more general, it was tested using another cell line. In the proliferation assay using LNCaP cell line, the analogue again gave results similar to PMA (Figure **1.56**). When Merle 32 was subjected to the attachment assay, once again, it behaved like PMA. In both of these assays involving LNCaP cells, Merle 32 was unable to block the effect of PMA in a dose dependent manner.

From all four assays involving both U937 and LNCaP cell lines, it is clear that the Merle 32 behaves like PMA. If the gem-dimethyl group was the group solely responsible for determining the bryo-like character, which might be inferred by the chemical and biological evaluation of the previous analogous, Merle 32 would have behaved like bryostatin 1. This result suggests that this is not the case and it can be said that there is no single group in the A-B region of bryostatin 1 that is singly responsible for switching between bryostatinand phorbol ester biological function.

Comparison of Various Bryostatin Analogues

The bryostatin analogues mentioned above have close structural relationships with bryostatin 1 and among themselves. Although they differ from the natural product at

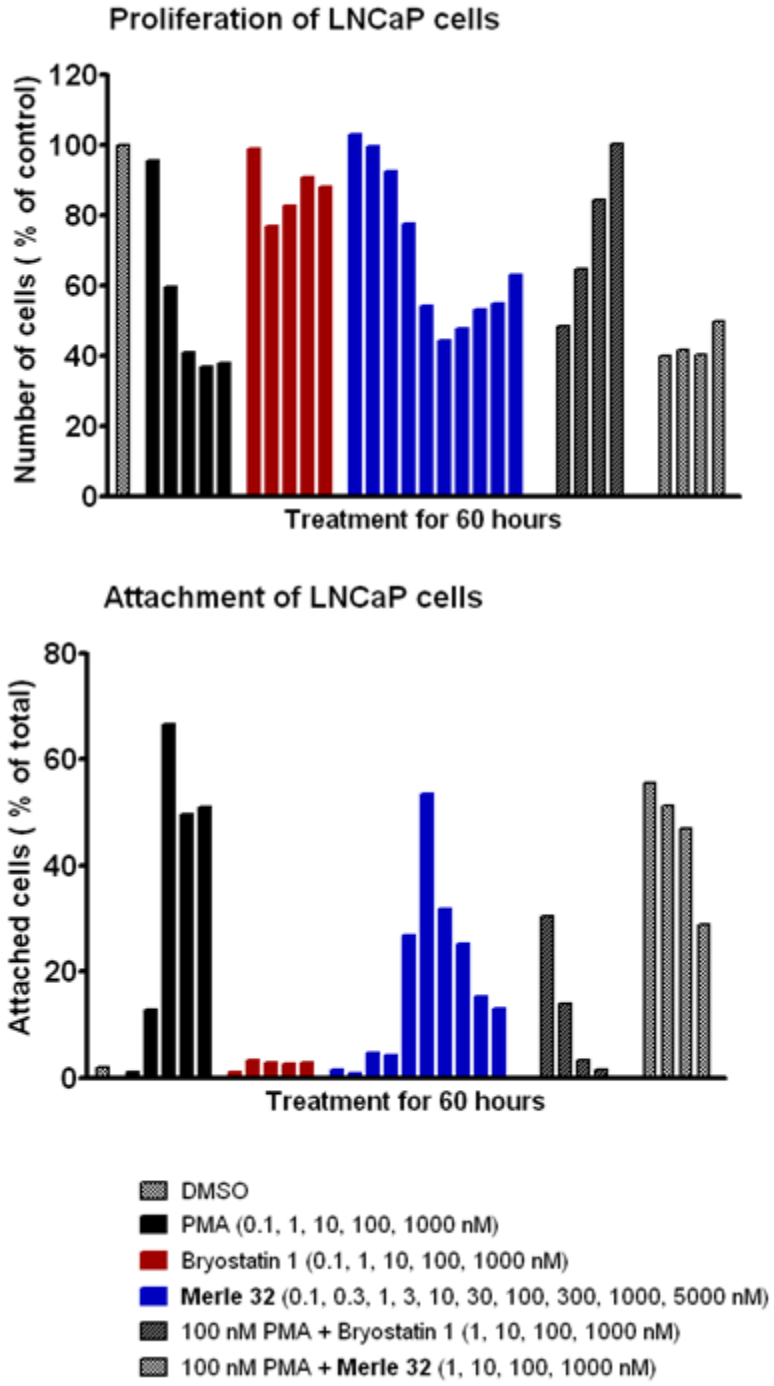


Figure 1.56. Assays Involving LNCaP Cell Lines

only a few positions, they have different biological properties. For example some analogues mimic the biological profile of bryostatin and are its agonists while others are antagonists. Even among the analogues which are functionally similar, their potency in functional responce is different. One of the goals of synthesizing various bryostatin analogues is to study the structure activity relationships and to ultimately optimize an analogue with respect to biological activity. Thus it is important to compare these analogues not only with the natural product, but also among themselves in terms of their biological activity.

Comparison of Merle 28 and Merle 30. As mentioned earlier, two of the analogues Merle 28 and Merle 30 are very close structural analogues of bryostatin 1. Merle 28 differs from bryostatin 1 at only one position. This analogue is devoid of the C_{30} carbomethoxy group present in the natural product. The other analogue Merle 30 lacks the C_9 hydroxyl group of bryoststin 1. When these analogues were tested for their binding affinity, both of them were found to have high binding affinity to PKCα. In fact, they have higher (Merle 28, K*i* = 0.52 nM, Merle 30 K*i* = 0.38 nM binding affinity than bryostatin 1 (K*i* = 1.35 nM) for purified PKCα. In order to determine the bryo or phorbol like behavior, both of these analogues were compared in cell proliferation and attachment assays using the U937 cell line (Figure **1.57**). In the proliferation assay, both of these analogues showed proliferation similar to bryostatin but different from that of PMA. In the attachment assay, they both induced the attachment characteristic of bryostatin 1. The biological behavior of these analogues was further tested in their ability to secret TNFα in LNCaP cell lines (Figure **1.58**). This assay revealed that both Merle 28 and 30 were similar to bryostatin but different from PMA. Thus this test verifies the general bryostatin

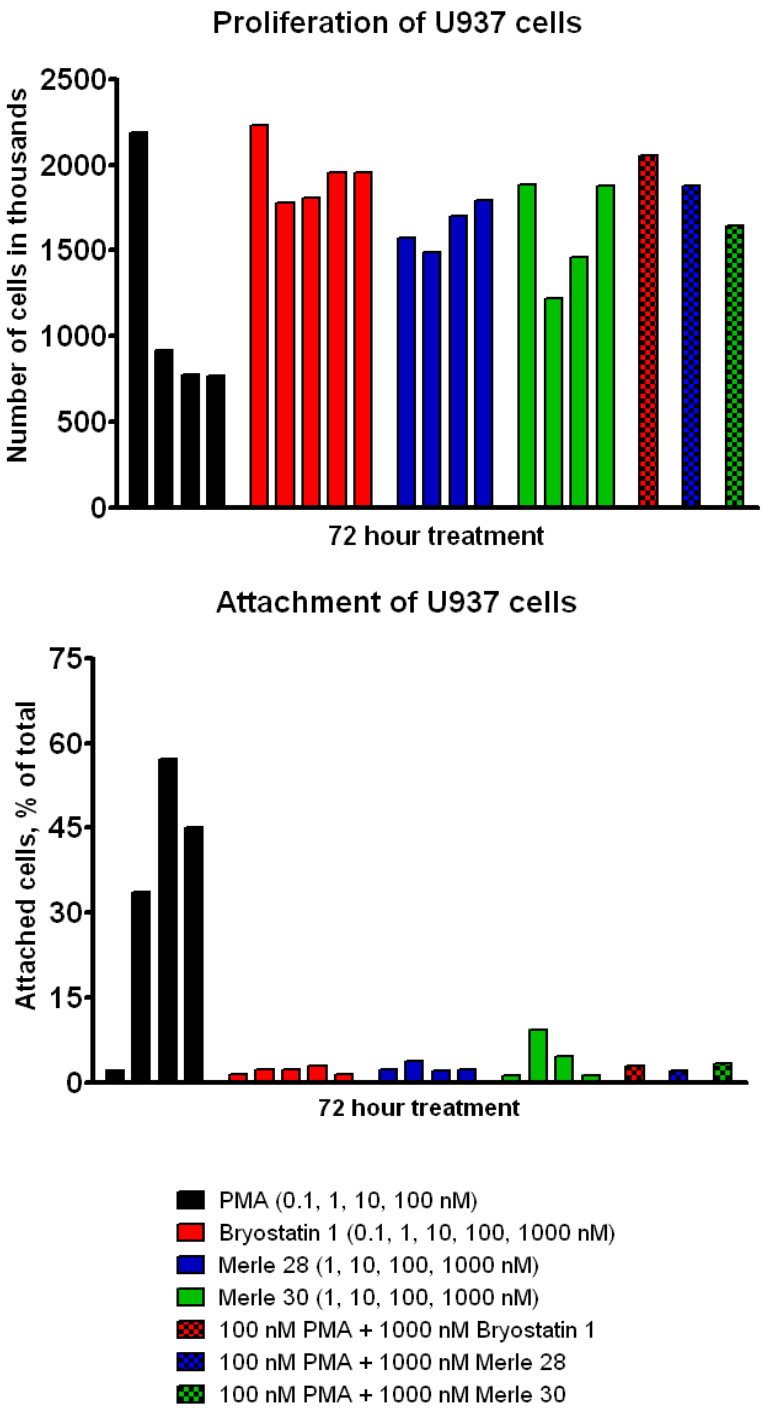


Figure 1.57. Comparison of Merle 28 and Merle 30

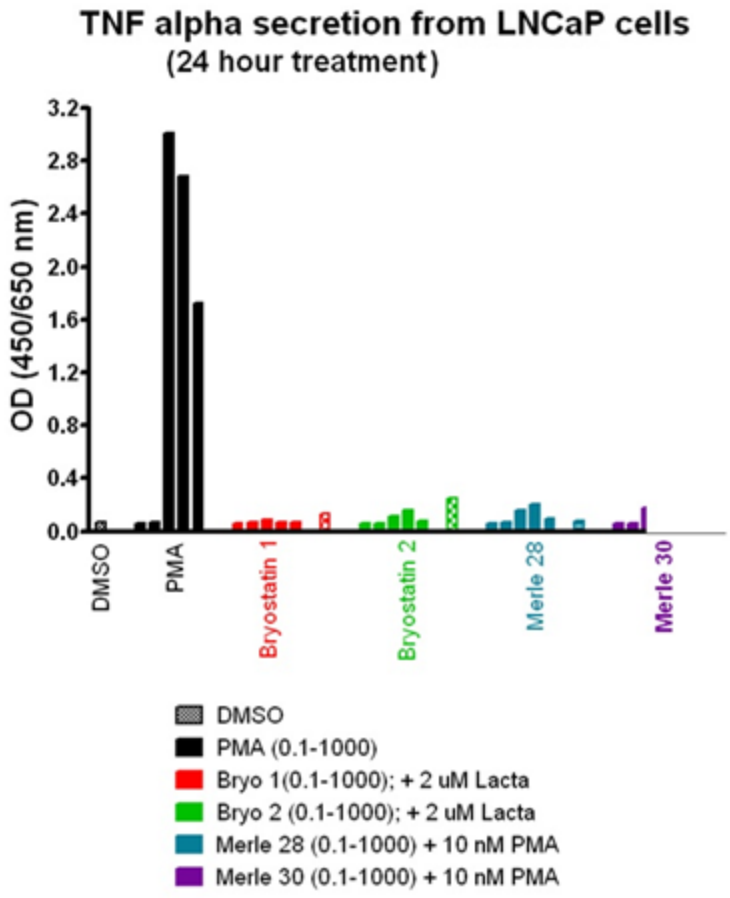


Figure 1.58. Comparison of Merle 28 and Merle 30

like biological profile of these analogues.

One interesting observation was made when the secretion of TNF-α by various bryostatin analogues was measured in the presence of Lactacystin. Lactacystin is a proteasome inhibitor which prevents the down-regulation of PKC. When the bryostatin analogues were tested with the proteasome inhibitor Lactacystin, the level of secreted TNF- α was significantly increased (Figure **1.59**). This suggests that analogues which behave otherwise like bryostatin can be reverted to more PMA-like compounds in the

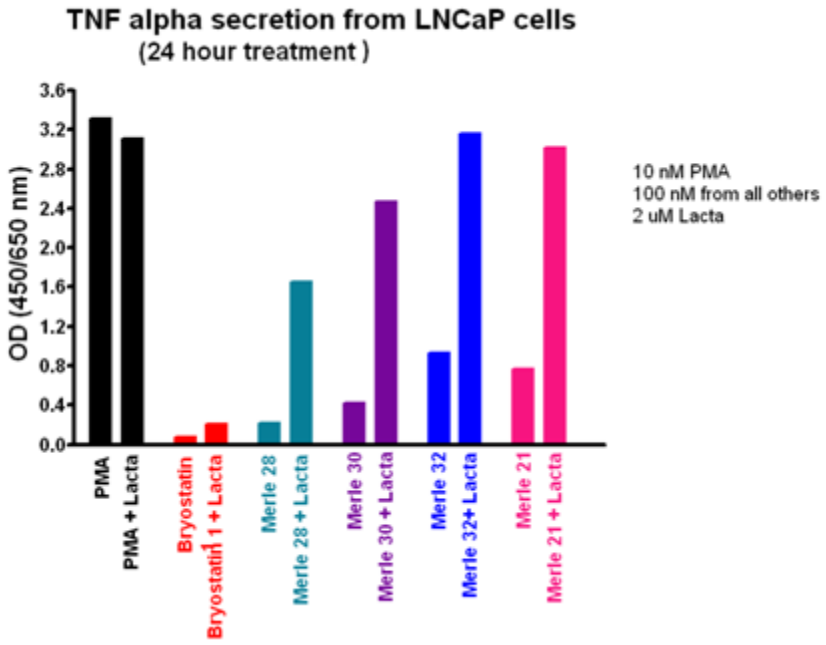


Figure 1.59. Reversal of the Biological Activity of Bryostatin Like Analogues

presence of lactacystin. The reason(s) for such switch in the biological effect is under investigation. Although both Merle 28 and Merle 30 were found to have similar higher binding affinity for PKCα to that of bryostatin 1, they were found to be slightly less potent than bryostatin 1 during attachment and proliferation assays. This can be seen by the larger decrease in the proliferation and greater attachment of the U937 cells by both of these analogues (Figure **1.57**). Between the two analogues, Merle 30 is slightly less potent than Merle 28. Such difference in the potency of the same compound in two different assays suggests that binding affinity and resulting biological function are different things.

Comparison of Merle 30 and Merle 32. Another comparison was made between Merle 30 and Merle 32. Merle 30 differs from bryostatin 1 at just one position whereas Merle 32 differs at three positions. As mentioned earlier, Merle 32 was prepared in order to reveal the role of the gem-dimethyl group on the A-ring. Merle 30 was found to be functional analogue of bryostatin 1, whereas Merle 32 was like PMA from the proliferation and attachment of the U937 and LNCaP cell lines. The biological function of these two analogous was further investigated using the MV4-11 cell line. The MV4-11 cell line is a human leukemia cell line that also exhibits differential response to various PKC ligands such as bryostatin and PMA. Both analogues were subjected to the proliferation and attachment assays. It is observed from the attachment assay that Merle 30 resulted in the attachment of the MV4-11 cell line similar to bryostatin 1 whereas the response shown by Merle 32 was like that of PMA (Figure **1.60**). Moreover, Merle 30 is able to block the effect of PMA in dose dependent manner whereas Merle 32 is not able to do so. In the proliferation assay, Merle 30 seems to have an effect intermediate between bryostatin 1 and PMA but Merle 32 is very similar to PMA. In this assay, Merle 30 is not able to block the effect of PMA as effectively as byrostatin 1. This suggests the dual nature of Merle 30 in some assays suggesting that Merle 30 is predominantly bryostatin 1 like but in some assays, it weakly resembles PMA.

Both of these compounds were further investigated in another human leukemia cell line, K562.[71] A proliferation assay involving this cell line suggested that Merle 32 behaves like PMA whereas Merle 30 displayes predominantly bryostatin 1 like character with little inclination towards PMA (Figure **1.61**). Both of these assays involving MV4-11 and K562 cell line further verify the behavior shown by Merle 30 and Merle 32 in the

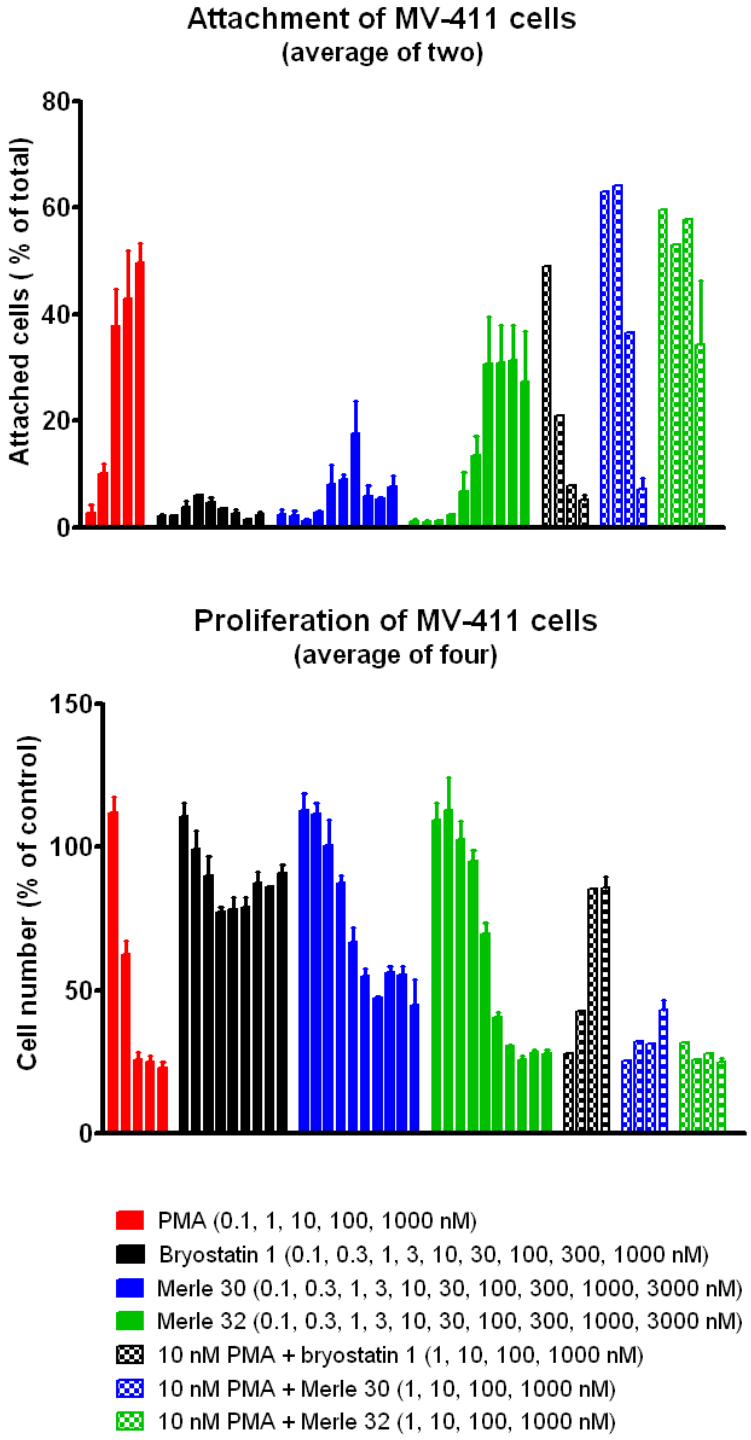


Figure 1.60. Comparison of Merle 30 and Merle 32

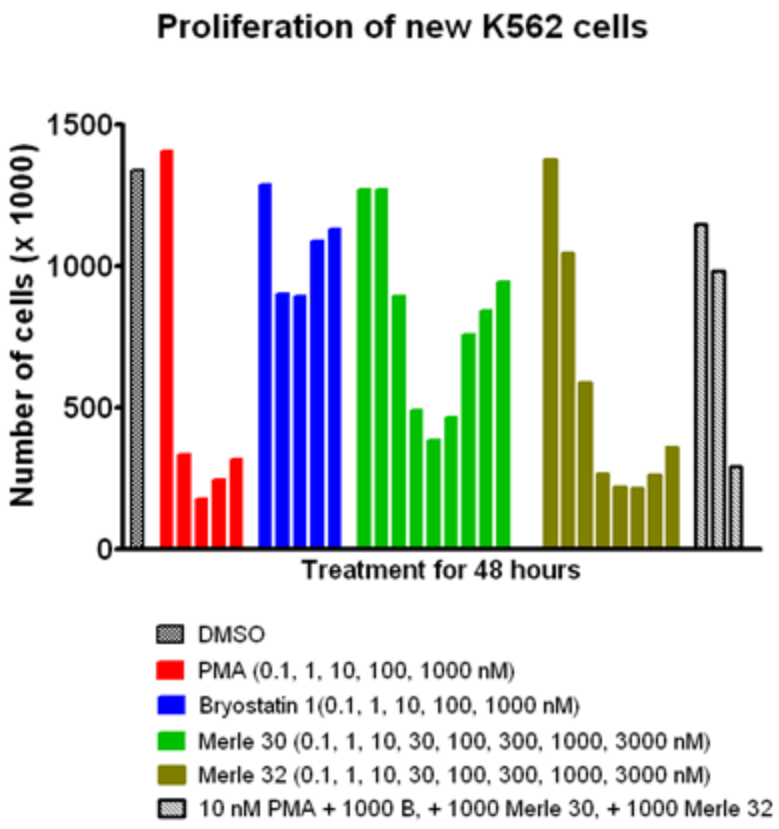


Figure 1.61. Comparison of Merle 30 and Merle 32

U937 and LNCaP cell lines that Merle 32 gave response similar to bryostatin 1 whereas Merle 30 is similar to PMA.

Comparison of Merle 23 and Merle 32. The comparison of Merle 32 is with another analogue Merle 23 which behaves like phorbol ester. The difference between these two analogues is at just one point, i.e., the presence of gem-dimethyl group on the A-ring of Merle 32. One of the reasons for synthesizing Merle 32 was that it could be most logically compared with Merle 23 the role of the gem-dimethyl group could be revealed. When these two analogues were compared in the cell attachment and

proliferation assays using the U939 cell line, both of them caused the attachment and proliferation of cells similar to PMA and thus behaved exactly similar to PMA (Figure **1.62**). Neither analogue were able to block the effect of PMA in dose dependent manner, a response characteristic of bryostatin. In fact Merle 23 displayed more PMA like response than Merle 32 in the attachment assay by inducing higher attachment of the U937 cells. This suggested that the presence or absence of the gem-dimethyl group on the C_8 position does not reverse the PMA-bryo like response.

Comparison of Merle 28 and Merle 32. A final comparison was made between the most bryostatin like analogue Merle 28 and PMA like Merle 32 (Figure **1.63**). The results showed that these two compounds behaved opposite to each other in terms of their PMA or bryo like character, Merle 28 was similar to bryostatin 1 whereas Merle 32 was just like PMA.

Translocation of PKCδ by bryostatin analogues. In addition to the cell attachment and proliferation assays, the biological behavior of various bryostatin analogues was also determined by examining the translocation of PKCδ by these compounds. As mentioned earlier, bryostatin is one of the potent exogeneous activators of PKC and most of the biological functions of the enzyme are attributed to its activation by various ligands. In addition to the endogenous activators such as DAG, PKC is also activated by a wide variety of nonendogenous ligands such as bryostatin and phorbol esters. It has been mentioned before that PKC activation and its translocation are related processes. The inactivated PKC is freely floating in the cytosol. Once PKC is activated by binding with a ligand such as bryostatin or PMA, its binding affinity with the lipid bilayer increases and it is translocated to the cell membrane. Once anchored to the membrane, the

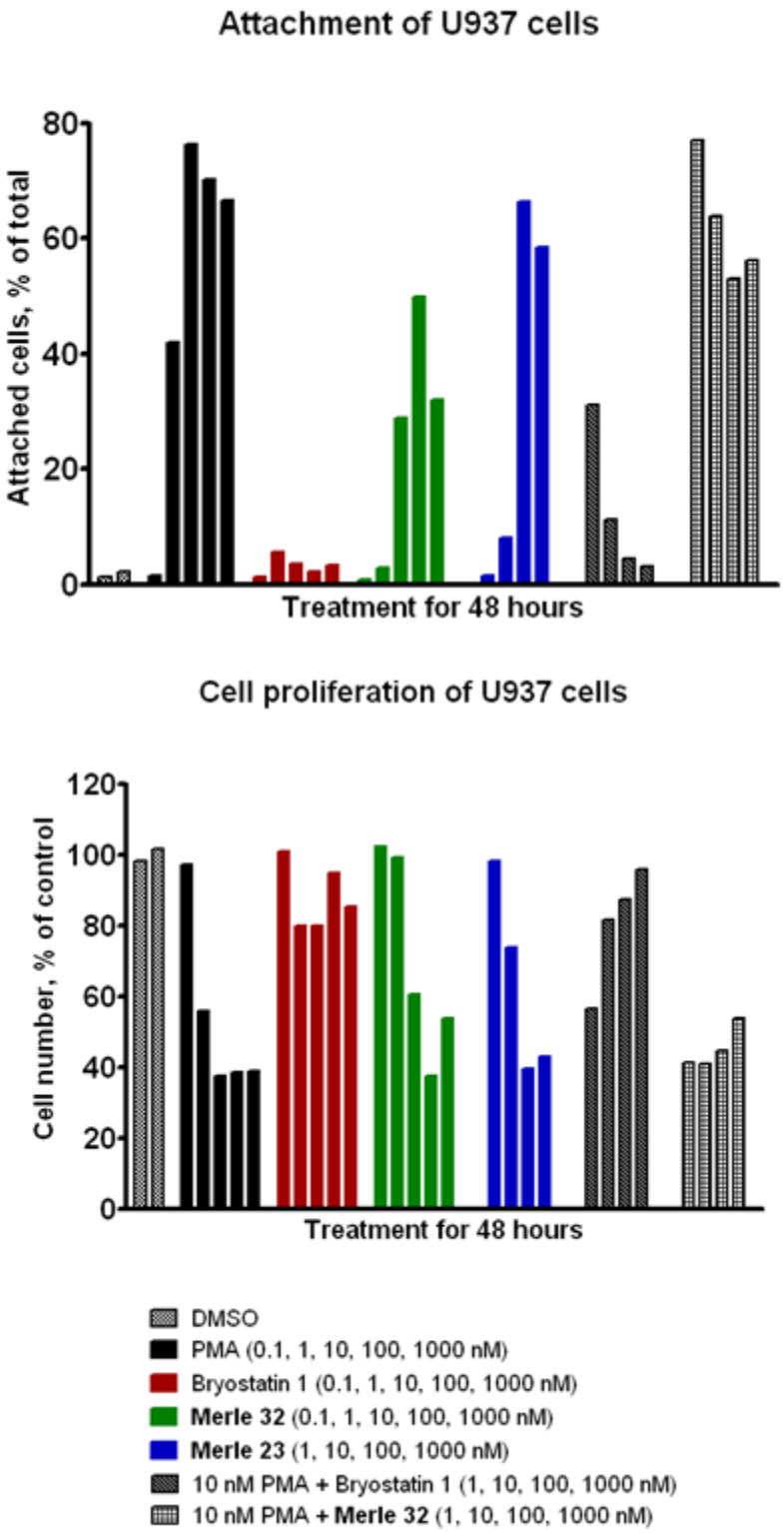


Figure 1.62. Comparison of Merle 23 and Merle 32

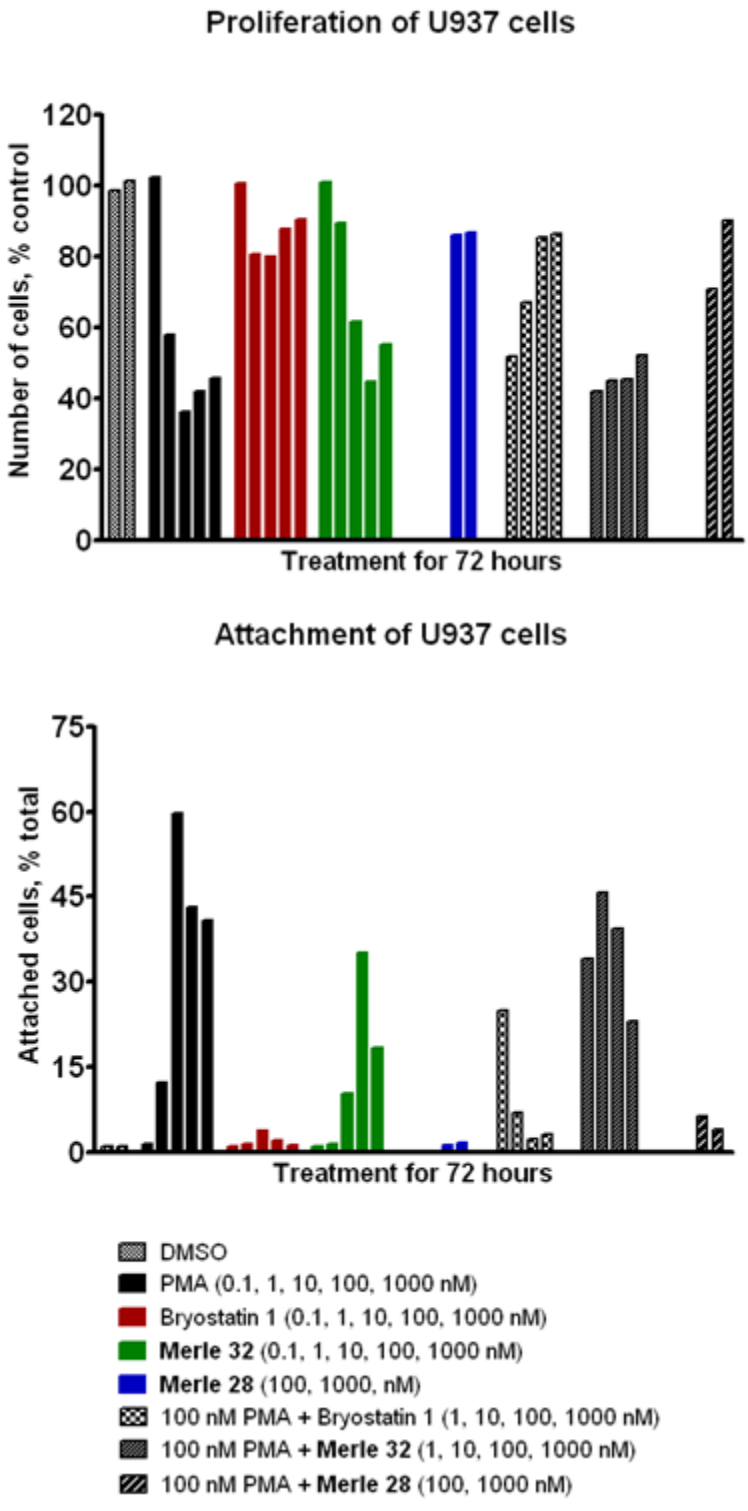


Figure 1.63. Comparison of Merle 28 and Merle 32

pseudosubstrate is removed from the substrate binding site of PKC leading to the phosphorylation of the target protein. It has been shown by Blumberg and coworkers that the pattern of translocation of PKCδ by various ligands is different.[72] Tumor promoters such as phorbol esters induce the translocation of PKC primarily to the plasma membrane. Bryostatin 1, a nontumor promoter, on the other hand, caused translocation of PKCδ primarily to the nuclear membrane. This opposite behavior of PMA and bryostatin towards PKC translocation thus serves as a tool to identify the PMA versus bryostatin like character of PKC ligands. The translocation of the PKCδ can be monitored in real time using PKC-green fluorescence protein (GFP) conjugate.

The preliminary results of PKCδ translocation assay in LNCaP cell using various bryostatin analogues is shown in Figure **1.64**. It can be observed from the figure that PMA shows strong membrane fluorescence due to the translocation of PKCδ leaving the cytosol almost empty. In contrast, bryostatin 1 shows no plasma membrane staining, some nuclear membrane and mostly cytoplasmic staining. A similar type of staining behavior is shown by Merle 28 indicating that biological function of Merle 28 is more similar to that of bryostatin but very different from that of PMA. On the other hand, Merle 30 shows more cell membrane staining than Merle 28 and bryostatin indicating its intermediate behavior which is also observed in the cell proliferation assay involving the MV4-11 cell line. In the case of Merle 23 and Merle 32, both show strong plasma membrane staining which is comparable to that of PMA indicating their PMA like nature. The observations made using translocation the assay are entirely consistent with the biological behavior shown by these analogues in the cell proliferation and attachment assays using various cell lines. Both of these assays point out that Merle 28 is a close

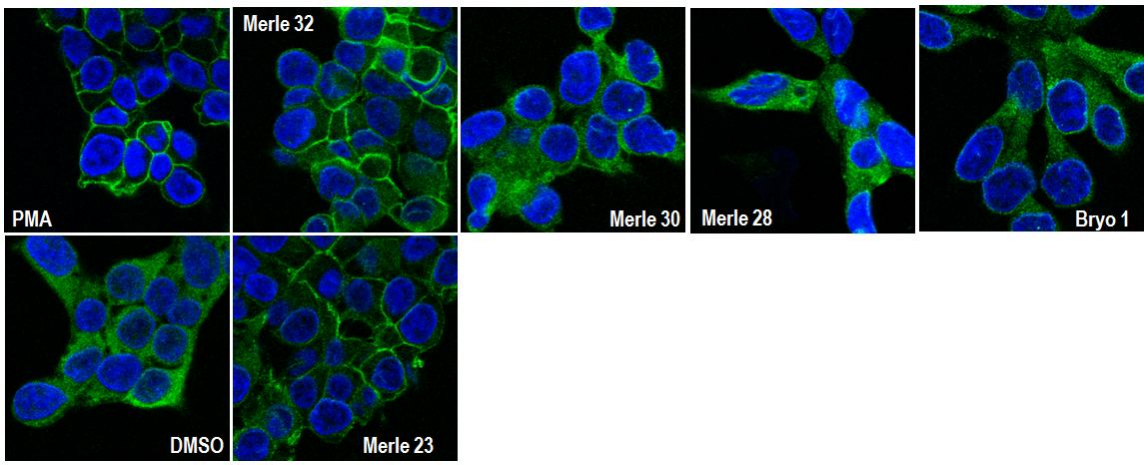


Figure 1.64. Translocation of Endogenous PKC-δ After 2 hr Treatment of LNCaP Cells

functional analogue of bryostatin 1 whereas Merle 23 and Merle 32, although both structurally similar to bryostatin 1, antagonize its effect and are functional analogues of PMA. Merle 30, on the other hand, shows more intermediate behavior between bryostatin and PMA.

The biological studies of various analogues using different assays revealed some interesting structure activity relationship of these compounds. Although all of these analogues have the basic bryostatin structure, their function varies from tumor promoting PMA to nontumor promoting bryostatin. Chemically, these analogues differ from each other only in the A-B-ring region. There are four different groups on the A-B region, C_7 acetate, C_8 gem-dimethyl, C_9 hydroxyl and C_{30} methyl ester. Three of the four groups are oxygen containing polar groups whereas the gem-dimethyl is more hydrophobic. Some analogues such as Merle 28 and Merle 30 have only one less polar group compared to bryostatin 1. Other compounds like Merle 23 and Merle 32 have all three polar groups removed from the A-B-ring region. It seems that the larger the number of polar groups on

the A-B-rings, the more bryostatin like the analogue is and vice versa. For example Merle 28, which has three polar groups (C_7 acetate, C_9 hydroxyl and C_{30} methyl ester), behaves like bryostatin 1 whereas Merle 32 in which all three polar groups are removed behaves like PMA. Between Merle 23 and Merle 32, everything is the same except the latter has an extra gem-dimethyl group on the A-ring which would make the A-B-ring region less polar. This suggests that Merle 32 would be more PMA like than Merle 23. In fact the opposite is true; the attachment assay using U937 cell shows that Merle 23 is more PMA like than Merle 32 (Figure **1.65**). This anomaly could arise due to the steric factor imposed by the extra gem-dimethyl group on Merle 32 that would make the interaction of A-B-ring region to the PKC different than without the gem-dimethyl group.

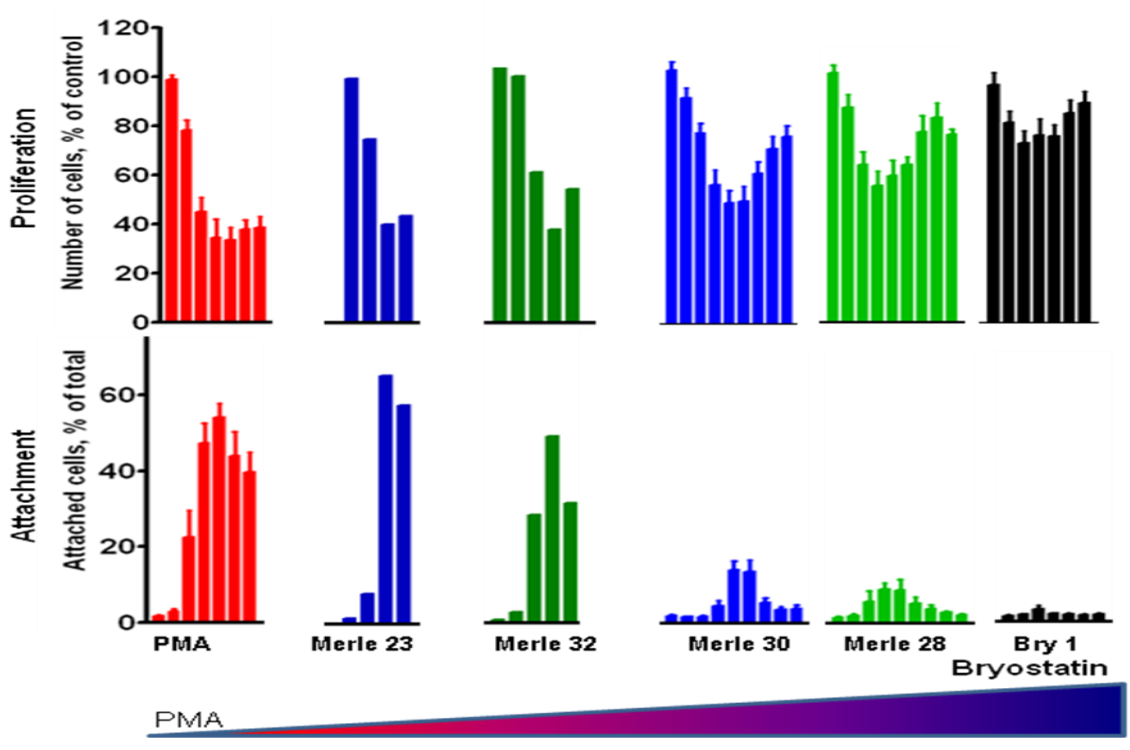


Figure 1.65. Comparison of Analogue Behavior on U937 Cell Line

When Merle 30 and Merle 28 are compared in terms of their A-B-ring polarity, Merle 30 has more polar group removed (assuming that a hydroxyl group is more polar than an ester group). Thus Merle 30 behaves less bryostatin 1 like and more PMA like than Merle 28 in the assays involving proliferation and attachment of various cell lines as well as the translocation assays. The polarity of a compound can be tentatively measured by calculating their ClogP values. The ClogP values of the A-B-ring region of various analogues and bryostatin are shown in Figure **1.66** calculated using ChemDraw. It is seen from the figure that the lower the ClogP value, the more bryostatin like activity and vice versa. Thus polarity can give a rough estimate of PMA-bryostatin like activity although polarity is by no means a single factor controlling the overall biological activity of these analogues.

Conclusions

In order to systematically study the structure activity relationship of bryostatin 1, three bryostatin analogues were prepared using the pyran annulation chemistry. Two of these analogues Merle 28 and Merle 30 differed from bryostatin at just one point, Merle 28 is C_{30}-decarbomethoxy bryostatin 1 whereas Merle 30 is C_9-deoxy bryostatin 1. The third analogue Merle 32 differs from PMA-like analogue Merle 23 at just the C_8 position with an additional gem-dimethyl group. The biological evaluation of these analogues was carried out in terms of their binding affinity towards PKC-α, their function in several differential assays using human cell lines. All these analogues showed binding affinity comparable to that of bryostatin 1 for PKC-α indicating that substitution at these three positions does not affect their ability to act as PKC ligands. Study of the biological

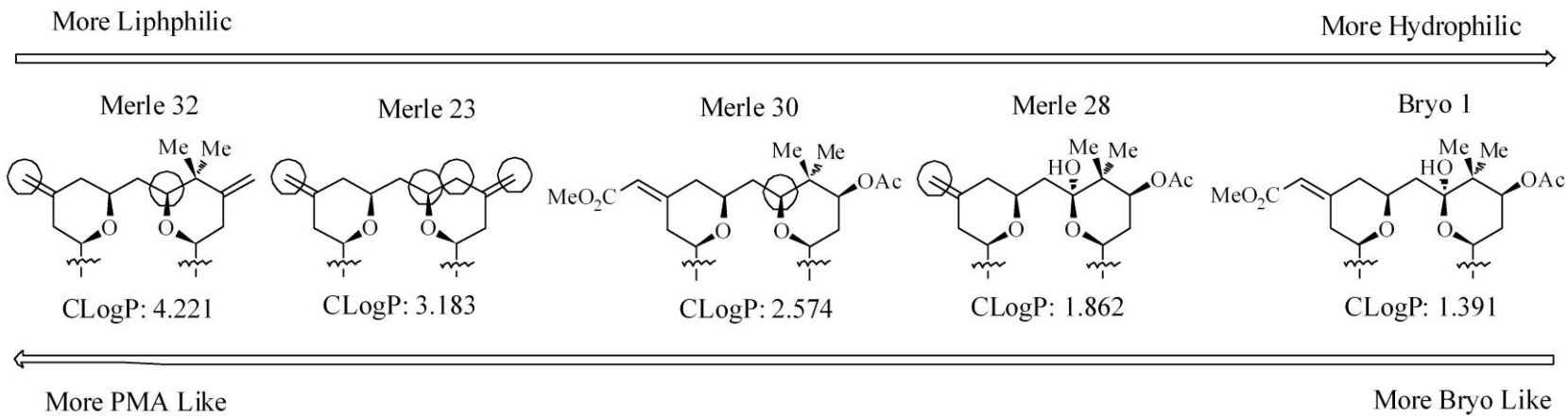


Fig 1.66. Comparison of Lipophilicity of Some Bryostatin Analogues

function revealed that Merle 28 and Merle 30 behave like bryostatin where Merle 32 was like PMA. These studies suggested that the C_{30} carbomethoxy group, C_9-OH or C_8 gem-dimethyl group do not alone switch the PMA versus bryostatin like activity. Thus the role of the three out of the four groups on the A-B region of the bryostatin 1 has been explored. A combination of four groups on the A-B-ring region (C_7 acetate, C_8 gem-dimethyl, C_9-OH, and C_{30} carbomethoxy) is ultimately responsible for the switch of PMA versus bryostatin like biology. The exact contributions of these groups have not been determined yet. However from the study of currently available analogues, it can be said that a combination that makes the A-B-ring region more hydrophilic leads to bryostatin 1 like behavior whereas the analogues with more lipophilic A-B-ring regions tend to behave like PMA.

Experimental Section

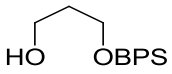
General Experimental Procedures, Materials, and Instrumentation

Solvents were purified according to the guidelines in *Purification of Common Laboratory Chemicals* (Perrin, Armarego, and Perrin, Pergamon: Oxford, 1966).[73] Diisopropylamine, diisopropylethylamine, pyridine, triethylamine, EtOAc, MeOH, and CH_2Cl_2 were distilled from CaH_2. The titer of *n*-BuLi was determined by the method of Eastham and Watson.[74] All other reagents were used without further purification. Yields were calculated for material judged homogenous by thin layer chromatography and nuclear magnetic resonance (NMR). Thin layer chromatography was performed on Merck Kieselgel 60 Å F_{254} plates or Silicycle 60Å F_{254} eluting with the solvent indicated, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 12-

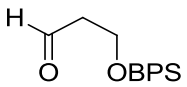
molybdophosphoric acid, or 4-anisaldehyde. Flash column chromatography was performed with Silicycle Flash Silica Gel 40 – 63 μm or Silicycle Flash Silica Gel 60 – 200 μm, slurry packed with 1% EtOAc/hexanes in glass columns. Preparative thin layer chromatography was performed on Silicycle 60Å F_{254} 20 cm × 20 cm × 250 μm plates. Glassware for reactions was oven dried at 125 °C and cooled under a dry nitrogen atmosphere prior to use. Liquid reagents and solvents were introduced by oven dried syringes through septum-sealed flasks under a nitrogen atmosphere. Nuclear magnetic resonance spectra were acquired at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts for proton nuclear magnetic resonance (^{1}H NMR) spectra are reported in parts per million relative to the signal of relative to the signal of residual $CHCl_3$ at 7.27 ppm. Chemical shifts for proton nuclear magnetic resonance (^{1}H NMR) spectra are reported in parts per million relative to the signal residual C_6D_6 at 7.16 ppm or $CDCl_3$ at 7.27 ppm . Chemicals shifts for carbon nuclear magnetic resonance (^{13}C NMR and DEPT) spectra are reported in parts per million relative to the center line of the C_6D_6 triplet at 128.39 ppm. Chemical shifts of the unprotonated carbons ('C') for DEPT spectra were obtained by comparison with the ^{13}C NMR spectrum. The abbreviations s, d, apd, dd, ddd, dddd, t, td, tt, q, dq, and m stand for the resonance multiplicity singlet, doublet, apparent doublet, doublet of doublets, doublet of doublet of doublets, doublet of doublet of doublet of doublets, triplet, triplet of doublets, triplet of triplets, quartet, doublet of quartets, and multiplet, respectively. Optical rotations (Na D line) were obtained using a microcell with 1 dm path length. Specific rotations ($[\alpha]_D^{20}$, Unit: °cm^2/g) are based on the equation $\alpha = (100 \cdot \alpha)/(l \cdot c)$ and are reported as unit-less numbers where the concentration c is in g/l00 mL and the path length l is in decimeters. Mass spectrometry was performed at the

mass spectrometry facility of the Department of Chemistry at The University of Utah on a double focusing high resolution mass spectrometer or at the mass spectrometry facility of the Department of Chemistry at the University of California, Riverside on an LCTOF mass spectrometer. Compounds were named using using ChemDraw 12.0.

Experimental Procedures and Analytical Data for Merle 28

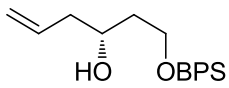


Preparation of 3-(*tert*-butyl-diphenyl-silanyloxy)-propan-1-ol (1.81). To the 1,3-propanediol (8.3 g, 109.16 mmol, 3 equiv), triethylamine (7.6 mL, 54.58 mmol, 1.5 equiv) and CH_2Cl_2 (200 ml) in a 500 ml flask at 0 °C was added *tert*-butyl(chloro)diphenylsilane (10.0 g, 36.8 mmol, 1 equiv), dropwise via syringe. The reaction was allowed to proceed for 12 h. The reaction was concentrated under reduced pressure and diluted with 30% EtOAc/Hexane (200 ml) and water (50 ml). Layers were separated and the organic layer was washed with brine (2 × 50 ml), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography of the crude product with 15% EtOAc/Hexane gave the desired product (8.25 g, 72% yield) as a white crystalline solid: mp = 40 °C, R_f = 0.37 (25% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70 – 7.68 (m, 4H), 7.46 – 7.39 (m, 6H), 3.87 – 3.84 (m, 4H), 2.36 (s, 1H), 1.82 (*J* = 5.67 Hz, 2H), 1.07 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 135.7, 133.4, 129.9, 127.9, 63.5, 62.2, 34.4, 27.0, 19.3.



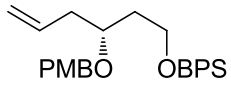
Preparation of 3-(*tert*-butyl-diphenyl-silanyloxy)-propion aldehyde (1.82). The stirring solution of oxalyl chloride (16.6 mL, 190.9 mmol**,** 1.5

equiv) and CH_2Cl_2 (500 mL) in a 1000 mL round bottom flask, was cooled to -78 °C and the mixture was allowed to stir for 10 min. Dimethyl sulfoxide (27.1 mL, 381.9 mmol**,** 3 equiv) was then added slowly via syringe to the reaction mixture and stirred for 10 min. The alcohol **1.81** (40.0 g, 127.3 mmol**,** 1 equiv) in 100 ml CH_2Cl_2 was cannulated to the reaction mixture slowly and an additional 10 ml CH_2Cl_2 was used for rinse. The reaction mixture stirred for 45 min at -78 °C and triethylamine (88.7 mL, 636.5 mmol**,** 5 equiv) was added via syringe to the reaction mixture. The reaction was allowed to proceed for 1 h and was allowed to warm up to 0 °C. The reaction was quenched by adding 100 ml saturated $NaHCO_3$ solution and 20% EtOAc/hexanes (200 ml). The layers were separated and organic layer was washed with brine (2 × 50 mL), water (2 × 50 ml), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product as a yellow oil. The crude product was purified by flash column chromatography with 10% EtOAc/hexanes which gave aldehyde **1.82** (38.1 g, 96% yield) as colorless oil: R_f = 0.40 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.84 (t, *J* = 2.19 Hz, 1H), 7.70 – 7.68 (m, 4H), 7.48 – 7.40 (m, 6H), 4.05 (t, *J* = 6.22 Hz, 2H) 2.63 (td, *J* = 6.2, 2.1 Hz, 2H), 1.07 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 202.0, 135.7, 133.4, 130.0, 127.0, 58.5, 46.6, 27.0, 19.38.

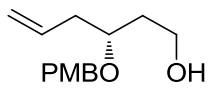


Preparation of (3*S*)-1-(*tert*-butyl-diphenyl-silanyloxy)-hex-5-en-3-ol (1.84). To 1000 mL round bottom flask was added a magnetic stir bar, oven dried 4 Å molecular sieves (51.2 g, 400 mg/mmol aldehyde), and CH_2Cl_2 (500 mL). To the stirring solution, was added (*S*)-BINOL (7.33 g, 25.60 mmol, 0.2 equiv) in one portion, a 1.0215 M solution of Ti(O*i*Pr)$_4$ (12.5 mL, 12.8 mmol, 0.1 equiv) in CH_2Cl_2 via syringe,

and a freshly prepared 0.1 M solution of TFA (8.9 mL, 0.89 mmol, 0.007 equiv) in CH_2Cl_2. The reaction mixture was heated at reflux (~40 °C) for 1 h, and then allowed to cool to rt. Aldehyde **1.82** (40.0 g, 128.0 mmol, 1.0 equiv), in CH_2Cl_2 (7 mL), was added to the reaction flask via cannula. An additional CH_2Cl_2 (5 mL) was for rinsing remaining aldehyde. The mixture was stirred for 0.5 h at rt, cooled to -78 °C and allyltributyltin (63.57 g, 192.0 mmol, 1.5 equiv) was added dropwise via syringe to the stirring mixture down the inside of the reaction flask. The reaction mixture was stirred for an additional 15 min at -78 °C then kept in a -20 °C freezer where it was briefly agitated every 24 h. After 5 days, the reaction mixture was filtered over a pad of Celite® into a stirring saturated aqueous $NaHCO_3$ solution (300 mL). The slurry was diluted with CH_2Cl_2 (200 mL) and the resulting mixture was stirred for 1 h and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with brine (2 × 100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product as red oil. Flash column chromatography of the crude product with 5% EtOAc/hexanes gave homoallyic alcohol **1.84** (40.16 g, 90% yield) as colorless oil: R_f = 0.46 (25% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70 – 7.69 (m, 4H), 7.47 – 7.40 (m, 6H), 5.87 (dddd, *J* = 17.2, 9.8, 6.9, 6.9 Hz, 1H), 5.16 – 5.12 (m, 2H), 4.01 – 4.3.96 (m, 1H), 3.93 – 3.83 (m, 2H), 3.23 (d, *J* = 2.4 Hz, 1H), 2.33 – 2.24 (m, 2H), 1.79 – 1.67 (m, 2H), 1.07 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 135.7, 135.1, 130.0, 130.0, 129.9, 117.5, 71.0, 63.4, 42.1, 38.0, 27.0, 19.2; Assay of enantiomeric excess: HPLC (Chiralcel OD-H 25 cm column, 2.5% *i*PrOH/hexanes; 0.5 mL/min); t_r (major) = 8.13 min, t_r (minor) = 8.92 min; 93% ee.

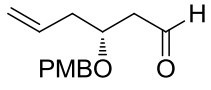


Preparation of *tert*-butyl-[(3*S*)-(4-methoxy-benzyloxy)-hex-5-enyl-oxy]-diphenylsilane (1.85). To a stirring solution of alcohol **1.84** (26.32 g, 74.65 mmol**,** 1.0 equiv) in CH_2Cl_2 (149 mL) in a 500 mL round bottom flask, was added freshly prepared 4-methoxybenzyl trichloroacetimidate (31.52 g, 111.97 mmol, 1.2 equiv). To the mixture was then added (±)-camphor-10-sulfonic acid (1.73 g, 7.465 mmol, 0.1 equiv) in one portion. The reaction was allowed to proceed for 12 h at rt, after which time TLC analysis indicated almost complete consumption of starting material. The reaction mixture was concentrated under reduced pressure, diluted with 20% EtOAc/hexanes (200 mL), filtered over a pad of Celite®, and concentrated under reduced pressure to give a red slurry. The crude product was purified by flash column chromatography eluting with 5% EtOAc/hexanes which gave PMB ether **1.85** (27.8 g, 79% yield) as colorless oil: R_f = 0.45 (20% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.71 – 7.68 (m, 4H), 7.47 – 7.39 (m, 6H), 7.24 – 7.22 (m, 2H), 6.87 – 6.86 (m, 2H), 5.87 (dddd, *J* = 17.2, 9.8, 6.9, 6.9 Hz, 1H), 5.13 – 5.08 (m, 2H), 4.47 (ABq, *J* = 10.9 Hz, Δν = 54.9 Hz, 2H), 3.88 – 3.83 (m, 1H), 3.81 (s, 3H), 3.80 – 3.73 (m, 2H), 2.35 (t, *J* = 6.0 Hz, 2H), 1.82 – 1.78 (m, 2H), 1.09 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.24, 135.77, 135.11, 134.13, 134.11, 131.15, 129.76, 129.48, 127.83, 117.15, 113.91, 75.28, 71.02, 60.73, 55.46, 38.75, 37.23, 27.11, 19.42.



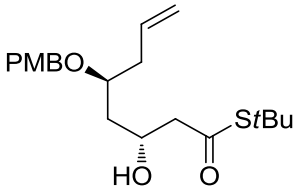
Preparation of (*S*)-3-(4-methoxy-benzyloxy)-hex-5-en-1-ol (1.86). To a stirring solution of BPS ether**1.85** (3.58 g, 7.54 mmol**,** 1.0 equiv) and THF (50 mL) in a 250 mL round bottom flask, at rt, was added a 1.0 M solution of tetrabutylammonium fluoride (9.42 mL, 9.42 mmol, 1.25 equiv) in THF, dropwise via

syringe. The reaction was allowed to proceed for 12 h at rt, after which the reaction mixture was concentrated under reduced pressure. The crude product was purified by flash column chromatography with 10% EtOAc/hexanes (400 mL), 25% EtOAc/hexanes (250 mL), and 50% EtOAc/hexanes (250 ml) which gave primary alcohol **1.86** (1.72 g, 97% yield) as colorless oil: R_f = 0.23 (50% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.27 – 7.25 (m, 2H), 6.89 – 6.87 (m, 2H), 5.82 (dddd, 17.2, 9.8, 6.9, 6.9 Hz, 1 H), 5.13 – 5.08 (m, 2H), 4.50 (ABq, *J* = 11.5 Hz, Δν = 81.7 Hz, 2H), 3.79 (s, 3H), 3.79 – 3.67 (m, 3H), 2.60 (s, 1H), 2.44 – 2.39 (m, 1H), 2.37 – 2.31(m, 1H), 1.81 – 1.71 (m, 2H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.39, 134.42, 130.46, 129.58, 117.62, 114.01, 77.51, 70.82, 60.72, 55.39, 38.21, 36.14;



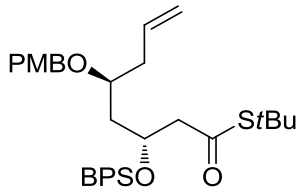
Preparation of (*S*)-3-(4-methoxy-benzyloxy)-hex-5-enal (1.74). To a stirring solution of alcohol **1.86** (5.0 g, 21.15 mmol**,** 1.0 equiv) and CH_2Cl_2 (211 mL) in a 500 mL round bottom flask, at -5 °C, was added freshly distilled *N*,*N*-diisopropylethylamine (26.6 mL g, 148.10 mmol, 7.0 equiv), dropwise via syringe. After 10 min at -10 °C, dimethyl sulfoxide (15.7 mL, 221.58 mmol, 10.0 equiv) was added to the reaction mixture via syringe and the solution was allowed to stir for an additional 10 min. Sulfur trioxide pyridine complex (13.47 g, 84.63 mmol, 4.0 equiv) was then added in one portion. The reaction was allowed to proceed for 30 min at -5 °C, was quenched by transferring the reaction mixture to a stirring saturated aqueous $NaHCO_3$ solution. The reaction mixture was then concentrated under reduced pressure; diluted with 25% EtOAc/hexanes (200 mL), and the layers were separated. The aqueous phase was extracted with 25% EtOAc/hexanes (4 × 50 mL). The combined organic layers were

washed with brine (2 × 50 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product as a pale yellow oil. The crude product was purified by flash column chromatography with 10% EtOAc/hexanes which gave aldehyde **1.84** (4.2 g, 85% yield) as colorless oil: R_f = 0.42 (40% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.74 (t, *J* = 2.44 Hz, 1H), 7.25 – 7.23 (m, 2H), 6.87 – 6.86 (m, 2H), 5.81 (dddd, *J* = 16.9, 9.9, 7.3, 7.3 Hz, 1H), 5.14 – 5.13 (m, 1H), 5.11 (s, 1H), 4.49 (ABq, *J* = 10.9 Hz, Δν = 47.8 Hz, 2H), 4.03 – 3.98 (m, 1H), 3.77 (s, 3H), 2.66 (ddd, *J* = 16.6, 7.8, 2.4 Hz, 1H), 2.55 (ddd, *J* = 16.6, 4.8, 1.9 Hz, 1H), 2.45 – 2.40 (m, 1H), 2.38 – 2.33 (m, 1H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 201.33, 159.28, 133.65, 130.19, 129.36, 118.17, 113.82, 73.30, 70.87, 55.22, 47.98, 38.31;



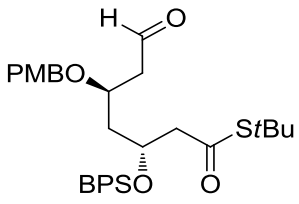
Preparation of (3*R*, 5*S*)-3-hydroxy-5-(4-methoxy-benzyloxy)-oct-7-enethioic acid *S*-*tert*-butyl ester (1.88). To a stirring solution of aldehyde **1.74** (3.98 g, 16.98 mmol, 1.0 equiv) and toluene (169 mL) in a 250 mL round bottom flask, at -78 °C, was added a freshly prepared 1.0 M solution of $TiCl_2(OiPr)_2$ (42.4 mL, 42.4 mmol, 2.5 equiv) dropwise via syringe. The resulting yellow solution was allowed to stir for 15 min, followed by dropwise addition of thioketene acetal **1.74** (9.03 g, 44.16 mmol, 2.6 equiv), in toluene (5 mL), down the inside of the reaction flask over a 5 min period. After 3 h, at -78 °C, the reaction was quenched by transferring directly into a vigorously stirring mixture of CH_2Cl_2 (200 mL) and pH 7.0 phosphate buffer (100 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with a saturated aqueous NH_4Cl solution (2 ×

100 mL), brine (2 × 100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a pale yellow oil. The crude product was purified by flash column chromatography with 10% EtOAc/hexanes which gave β-hydroxy thiol ester **1.88** (5.79 g, 93% yield) as colorless oil: R_f = 0.42 (25% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.27 – 7.26 (m, 2H), 6.88 – 6.86 (m, 2H), 5.79 (dddd, *J* = 17.0, 10.2, 6.8, 6.8 Hz, 1H), 5.11 – 5.06 (m, 2H), 4.50 (ABq, *J* = 10.7 Hz, Δν = 69.9 Hz, 2H), 4.29 – 4.28 (m, 1H), 3.79 (s, 3H), 3.79 – 3.76 (m, 1H), 3.18 (s, 1H), 2.60 – 2.58 (m, 2H), 2.43 – 2.38 (m, 1H), 2.35 (ddd, *J* = 14.0, 7.0, 7.0 Hz, 1H), 1.67 – 1.56 (m, 2H), 1.46 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 200.0, 159.4, 134.4, 130.5, 129.6, 117.6, 114.0, 75.4, 71.2, 65.9, 55.4, 51.5, 48.5, 40.2, 38.3, 29.9.



Preparation of (3*R*, 5*S*)-3-(*tert*-butyl-diphenyl-silanyloxy)-5-(4-methoxy-benzyloxy)-oct-7-enethioic acid *S*-*tert*-butyl ester (1.89). To a stirring solution of a β-hydroxy thiol ester **1.88** (1.00 g, 2.72 mmol**,** 1.0 equiv), imidazole (0.55 g, 8.18 mmol, 3.0 equiv), and DMF (9.0 mL) in a 15 mL round bottom flask, at rt, was added *tert*-butyl(chloro)diphenylsilane (0.89 g, 3.27 mmol, 1.2 equiv) via syringe. The reaction was allowed to proceed for 24 h, and was quenched by adding a mixture of 10% EtOAc/hexanes (100 mL) and H_2O (10 mL) in a rb flask. The layers were separated and the organic layer was washed with brine (2 × 50 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography with 5% EtOAc/hexanes which gave silyl ether **1.89** (1.6 g, 96% yield) as a pale yellow oil: R_f = 0.27 (5% EtOAc/hexanes); 500 MHz

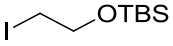
^{1}H NMR ($CDCl_3$) δ 7.71 – 7.67 (m, 4H), 7.42 – 7.34 (m, 6H), 7.11 – 7.09 (m, 2H), 6.82 – 6.81 (m, 2H), 5.58 (dddd, *J* = 17.5, 10.7, 7.3, 7.3 Hz, 1H), 4.96 – 4.92 (m, 2H), 4.38 (tt, *J* = 6.3, 5.8 Hz, 1H), 4.28 (d, *J* = 10.74 Hz, 1H), 4.02 (d, *J* = 10.7 Hz, 1H), 3.79 (s, 3H), 3.35 – 3.31 (m, 1H), 2.71 (dd, *J* = 14.6, 6.3 Hz, 1H), 2.65 (dd, *J* = 14.6, 6.6 Hz, 1H), 2.11 – 2.02 (m, 2H), 1.76 (ddd, *J* = 14.3, 8.4, 5.9 Hz, 1H), 1.66 (ddd, *J* = 14.3, 6.2, 3.9 Hz, 1H), 1.42 (s, 9H), 1.03 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 197.9, 159.1, 136.1, 136.1, 134.0, 134.5, 134.2, 131.0, 129.8, 129.7, 129.3, 127.8, 127.7, 117.2, 113.8, 75.7, 70.2, 69.1, 55.4, 53.0, 48.1, 42.4, 38.5, 29.9, 27.1, 19.6.



Preparation of (3*R*, 5*S*)-3-(*tert*-butyl-diphenyl-silanyloxy)-5-(4-methoxy-benzyloxy)-oct-7-oxo-heptanethioic acid *S*-*tert*-butyl ester (1.72). To a stirring solution of olefin **1.89** (3.0 g, 4.95 mmol**,** 1.0 equiv), THF (23 mL), *t*-butanol (22.5 mL), and H_2O (4.5 mL) in a 100 mL round bottom flask, was added 4-methylmorpholine *N*-oxide (726 mg, 3.19 mmol, 1.25 equiv) in one portion. A 0.078 M solution of OsO_4 (3.14 mL, 0.247 mmol, 0.05 equiv) in THF was added dropwise, via syringe. The reaction was allowed to proceed for 12 h, and was quenched by addition of sodium metabisulfite ($Na_2S_2O_5$) in one portion and the mixture was stirred for 1 h, during which time color changed from yellow to dark brown. The reaction mixture was diluted with H_2O (50 mL), EtOAc (200 mL), and the layers were separated. The aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with brine (2 × 25 mL), dried over $MgSO_4$, filtered, concentrated under reduced pressure give

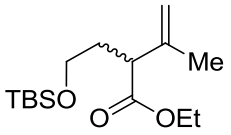
crude diol **1.90** (3.22 g) as a thick paste that was taken directly onto the next reaction without further purification.

To a stirring solution of crude diol **1.90** (3.22 g, approx. 5.03 mmol, 1.0 equiv) and benzene (50 mL), at rt, was added $Pb(OAc)_4$ (2.34 g, 5.29 mmol, 1.05 equiv) in five portions over 10 min. After 2 h, the reaction mixture was diluted with hexanes (200 mL), filtered over a pad of Celite® and Na_2SO_4, and concentrated under reduced pressure to yield crude aldehyde. The crude product was purified by flash column chromatography with 10% EtOAc/hexanes which gave aldehyde **1.72** (2.91 g, 95% yield over 2 steps) as a viscous colorless oil: R_f = 0.40 (20% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.54 (s, 1H), 7.74 – 7.70 (m, 4H), 7.46 – 7.37 (m, 6H), 7.11 – 7.09 (m, 2H), 6.84 – 6.83 (m, 2H), 4.44 (tt, *J* = 5.8, 5.6 Hz, 1H), 4.20 ABq, *J* = 10.7 Hz, Δν = 42.6 Hz, 2H), 3.84 – 3.82 (m, 1H), 3.80 (s, 3H), 2.73 (dd, *J* = 14.6, 5.8 Hz, 1H), 2.64 (dd, *J* = 14.6, 6.3 Hz, 1H), 2.34 (ddd, *J* = 16.1, 6.3, 1.9 Hz, 1H), 2.27 (ddd, *J* = 16.5, 4.9, 2.3 Hz, 1H), 1.97 (ddd, *J* = 13.6, 6.8, 6.8 Hz, 1H), 1.65 (ddd, *J* = 14.1, 5.8, 5.8 Hz, 1H), 1.44 (s, 9H), 1.06 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 201.0, 197.6, 159.3, 136.1, 136.0, 133.8, 133.6, 130.2, 130.0, 129.9, 129.4, 127.8, 113.8, 71.3, 70.6, 68.4, 55.4, 52.5, 48.5, 48.2, 42.7, 29.9, 27.1, 27.1, 19.5.



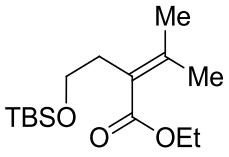
Preparation of *tert*-butyl-(2-iodo-ethoxy)-dimethyl silane (1.74). To the commercially available 2-iodoethanol (20.0g, 116.29 mmol, 1.0 equiv) in a 500 ml flask were added CH_2Cl_2 (233 ml) and imidazole (23.75 g, 348.87 mmol, 3 equiv). To this solution was then added TBSCl (18.4g, 122.1 mmol, 1.05 equiv) in one portion. The mixture was stirred at room temperature for 24 hr after which it was diluted with 250 ml of hexane and 100 ml of water. The layers were separated and the organic layer was

washed with brine (2 × 50 ml), dried over $MgSO_4$, concentrated under reduced pressure. Flash column chromatography with 2% EtOAc/Hexane gave the desired compound (32.63 g, 98% yield) as colorless oil; R_f = 0.40 (2.5% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 3.84 (t, *J* = 6.9, 2H), 3.20 (t, *J* = 7.3, 2H), 0.91 (s, 9H), 0.09 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 64.4, 26.0, 18.53, 7.2, -4.9.



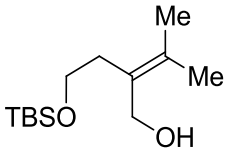
Preparation of 2-[-*tert*-butyl-dimethyl-silanyloxy-ethyl]-3-methyl-but-3-enoic acid ethyl ester (1.91). To a flame dried 500 ml flask was added diisopropylamine (6.6 mL, 46.8 mmol, 1.1 equiv) in THF (229 ml) and the mixture was cooled to 0 °C. To the solution was added a solution of *n*-BuLi in hexanes (17.3 ml of 2.48 M, 42.91 mmol, 1.1 equiv) dropwise via syringe. The pale yellow solution was stirred at 0 °C for 30 min and cooled to -78 °C. Ethyl 2, 2-dimethyl acrylate (5.0 g, 39 mmol, 1 equiv) was then added dropwise via syringe to the reaction mixture. After 30 min, a solution of iodide **1.74** (11.16 g, 39 mmol, 1 equiv) in THF (5 ml) was added dropwise to the reaction mixture via cannula. The reaction was allowed to proceed for 12 hr during which time the -78 °C bath came to rt. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution (50 mL) and the mixture was diluted with 100 ml of water and 200 ml of ether. The layers were separated and aqueous layer was extracted with diethyl ether (2 × 50 ml). The combined organic layers were washed with brine (2 × 50 ml), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatographed with 1% EtOAc/hexane (1 liter), 2.5% EtOAc/hexane (1 liter), and 3.5% EtOAC/hexane (1 liter) to give the

desired product (8.95 g, 80% yield) as a colorless oil. R_f = 0.40 (5% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 4.90 (m, 1H), 4.89 (m, 1H), 4.14 (dq, *J* = 7.3, 3.6 Hz, 2H), 3.59 (t, *J* = 6.2 Hz, 2H), 3.25 (t, *J* = 7.6 Hz, 2H), 2.06 (dddd, *J* = 13.9, 8.1, 6.2, 6.2 Hz, 1H), 1.75 (s, 3H), 1.25 (t, *J* = 6.9 Hz, 3H), 0.89 (s, 9H), 0.04 (s, 6H),); 125 MHz ^{13}C NMR ($CDCl_3$) δ 173.8, 142.6, 113.9, 60.8, 60.7, 49.4, 33.2, 26.1, 20.5, 18.5, 14.3, -5.1, -5.2.

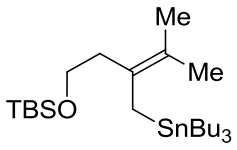


Preparation of 2-[2-(*tert*-Butyl-dimethyl-silanyloxy)-ethyl]-3-methyl-but-2-enoic acid ethyl ester (1.92). An oven-dried 25 mL round bottom flask was charged with alkene **1.91** (1.0 g, 3.49 mmol, 1.0 equiv) a magnetic stir bar, and then was purged with N_2 for 15 min. THF (7 mL), which was purged with N_2 for 30 min, was added to the reaction flask via Gastight® syringe. After cooling the mixture to 0 °C, potassium *tert*-butoxide (0.391 g, 3.49 mmol, 1.0 equiv), was added to the mixture in one portion under the stream of N_2. The reaction was allowed to proceed for 2 h at 0 °C in which time TLC showed the completion of the reaction. The reaction was quenched by transferring into a flask that contained a stirring mixture of a saturated aqueous NH_4Cl solution (25 mL) and Et_2O (100 mL). The layers were separated and the organic layer was washed with brine (2 × 20 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography, eluting with 5% EtOAc/hexanes, providing ester **1.92** (0.952 g, 95% yield) as a colorless oil: R_f = 0.31 (10% EtOAc/hexanes); 300 MHz ^{1}H NMR ($CDCl_3$) δ 4.18 (q, *J* = 7.0 Hz, 2H), 3.64 (t, *J* = 6.8 2H), 2.56 (t, *J* = 7.3 Hz, 2H), 2.00 (s, 3H), 1.85 (s, 3H), 1.30 (t, *J* =

6.7 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.7, 145.3, 124.3, 62.4, 60.2, 33.7, 26.1, 22.5, 23.3, 18.5, 14.5, –5.1.

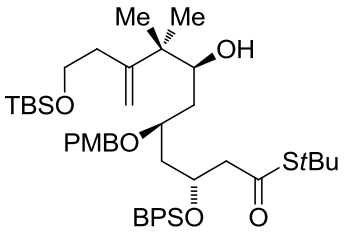


Preparation of 2-[2-(*tert*-butyl-dimethyl-silanyloxy)-3-methylbut-2-en-1-ol (1.93). To a stirring solution of the ester **1.92** (1.0 g, 3.49 mmol, 1 equiv) in CH_2Cl_2 (35 ml) in a 100 ml flask at 0 °C was added a solution of diisobutylaluminium hydride (8.72 ml of 0.1M, 8.72 mmol, 2.5 equiv) in CH_2Cl_2 dropwise via syringe. The mixture was allowed to proceed for 2 h at 0 °C at which time TLC showed the completion of the reaction. The reaction was quenched by dropwise addition of water and the mixture was transferred to a flask that contained a vigorously stirring solution of CH_2Cl_2 (25 ml) and saturated aqueous solution of potassium sodium (25 ml). The mixture was stirred vigorously for overnight and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 25 ml). The combined organic layers were washed with brine (2 x 20 ml), dried over $MgSO_4$, concentrated under reduced pressure. Purification was accomplished using flash column chromatography, eluting with 10% EtOAc/hexanes, providing allylic alcohol (721 mg, 91% yield) as a pale yellow oil: R_f = 0.35 (20% EtOAc/hexanes); 500 MHz ^1H NMR ($CDCl_3$) δ 4.10 (d, *J* = 5.6 Hz, 2H), 3.70 (t, *J* = 5.7 Hz, 2H), 3.24 (t, *J* = 5.6 Hz, 1H), 2.42 (t, *J* = 5.95 Hz, 2H), 1.76 (s, 3H), 1.1.70 (s, 3H), 0.90 (s, 9H), 0.08 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 130.8, 130.3, 63.3, 63.1, 34.6, 26.1, 20.7, 20.4, 18.5, –5.2.



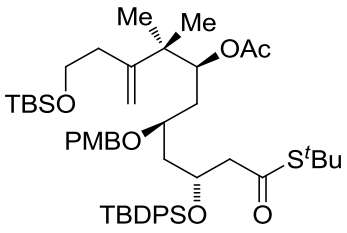
Preparation of *tert*-butyl-dimethyl-(3-methyl-tributyl-stannanyl-methyl-pent-enyloxy)-silane (1.71). To a stirring solution of allylic alcohol **1.93** (1.62 g, 7.54 mmol, 1.0 equiv) in THF (7 mL) in a flame-dried 50 mL round bottom flask, at -78 °C, was added a solution of *n*-BuLi (3.38 mL of 2.45 M, 8.3 mmol, 1.1 equiv) in hexanes dropwise via syringe. The resulting yellow solution was stirred for 15 min at -78 °C. Separately, to a stirring solution of diisopropylamine (2.6 mL, 18.9 mmol, 1.1 equiv) and THF (7 mL) in a 25 mL round bottom flask, at 0 °C, was added a solution of *n*-BuLi (6.5 mL of 2.45 M, 15.85 mmol, 2.1 equiv) in hexanes dropwise via syringe. After 45 min, tributyltin hydride (4.4 mL, 15.09 mmol, 1.0 equiv) was added via syringe to the freshly prepared LDA solution. After 15 min, methanesulfonyl chloride (1.1 g, 7.54 mmol, 1.0 equiv) was added to the lithium alkoxide solution at -78 °C. After 1.25 h, the Bu_3SnLi solution was added dropwise to the reaction flask via cannula. An additional THF (1 mL) rinse was used to transfer Bu_3SnLi residue. The reaction was allowed to proceed for 2 h at -78 °C, then for an additional 12 h during which time the -78 °C bath expired to reach rt. The reaction mixture was quenched by adding water (15 ml), diluted with 10% EtOAc/hexanes (150 mL) and layers separated. The organic layer was washed with brine (2 × 10 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography with hexanes providing allyl stannane **1.71** (2.81 g, 71% yield) as a colorless oil: R_f = 0.20 (hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 3.62 (t, *J* = 8.0 Hz, 2H), 2.20 (t, *J* = 7.6 Hz, 2H), 1.72 (s, 1H), 1.67 (s, 3H), 1.59 (s, 3H), 1.51 – 1.44 (m, 6H), 1.34 – 1.27 (m, 6H), 0.91 (s, 9H),

0.90 – 0.88 (m, 9H), 0.85 – 0.82 (m, 6H), 0.07 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) 128.3, 120.6, 62.5, 38.7, 29.4, 27.6, 26.2, 21.0, 20.3, 18.6, 17.2, 13.9, 10.0, –4.9.



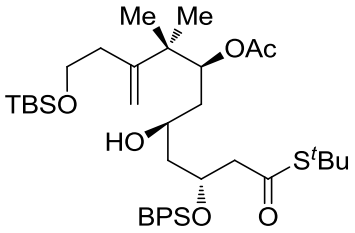
Preparation of 11-(*tert*-butyl-dimethyl-silanyloxy)-3-(*tert*-butyl-diphenyl-silanyloxy)-7-hydroxy-5-(4-methoxy-benzyloxy)-8,8-dimethyl-9-methylene-undecanethioic acid S-*tert*-Butyl ester (1.94). To a stirring solution of aldehyde **1.72** (300 mg, 0.49 mmol, 1.0 equiv) in toluene (4 mL) in a 15 mL round bottom flask, at -78 °C, was added a freshly prepared solution of Me_2AlCl (823 μL of 3.0 M, 2.47 mmol, 5.0 equiv) in toluene dropwise via syringe. The solution was stirred for 5 min at -78 °C, then stannane **1.71** (332.0 mg, 0.642 mmol, 1.3 equiv) in toluene (900 μL) was added dropwise, via syringe, down the inside of the reaction flask. The reaction was allowed to proceed for 2 h at -78 °C, and then quenched by transferring into a vigorously stirring mixture of a saturated aqueous potassium sodium tartrate solution (25 mL) and 20% EtOAc/hexanes (50 mL). The resulting mixture was allowed to stir for 2 h and then the layers were separated. The aqueous layer was extracted with 20% EtOAc/hexanes (2 × 25 mL). The combined organic layers were washed with brine (2 × 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the crude product as a pale yellow oil. Purification was accomplished by flash column chromatography eluting with 5% EtOAc/hexanes providing coupled product **1.94** (315 mg, 76% yield) as a single diastereomer by NMR; R_f = 0.58 (25% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.73 – 7.69 (m, 4H), 7.44 – 7.36 (m, 6H), 7.13 – 7.12 (m, 2H), 6.83 – 6.81 (m, 2H),

4.97(s, 1H), 4.90 (s, 1H), 4.31 (quin, *J*=5.9 Hz, 1H), 4.21 (ABq, *J* = 11.6 Hz, Δν = 50.0 Hz, 2H), 3.80 – 3.75 (m, 2H), 3,79 (s, 3H), 3.68 (d, *J* = 9.5 Hz, 1H), 3.64 – 3.59 (m, 1H), 2.69 (dd, *J* = 14.6, 5.8 Hz, 1H), 2.63 (dd, *J* = 14.6, 5.8 Hz, 1H), 2.40 (d, *J* = 2.93 Hz, 1H), 2.28 (ddd, *J*= 15.0, 7.3, 7.3 Hz, 1H), 2.22 (ddd, *J*= 15.0, 6.2, 6.2 Hz, 1H), 1.94 (td, *J* = 13.9, 6.6 Hz, 1H), 1.57 (td, *J* = 13.9, 5.6 Hz, 1H), 1.44 (s, 9H), 1.26 – 1.16 (m, 2H), 1.04 (s, 9H), 0.94 (s, 3H), 0.90 (s, 9H), 0.87 (s, 3H), 0.10 (s, 3H), 0.06 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 197.8, 159.2, 151.8, 136.1, 136.1, 134.1, 133.9, 130.9, 129.9, 129.8, 129.5, 127.8, 113.8, 111.2, 74.4, 71.6, 71.0, 68.9, 63.5, 55.4, 52.3, 48.1, 43.8, 42.6, 35.5, 34.0, 30.0, 27.1, 26.2, 22.7, 21.5, 19.5, 18.6, -5.0, -5.1.



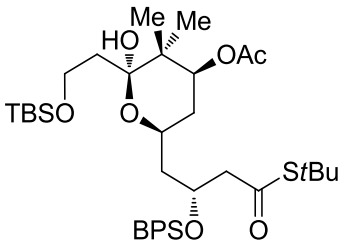
Preparation of acetic acid 1-[4-(*tert*-butyl-dimethyl-silanyloxy)-1,1-dimethyl-2 methylene-butyl]-5-(*tert*-butyl-diphenyl-silanyloxy)-6-*tert*-butylsulfanylcarbonyl-3- -(4-methoxy-benzyloxy)-hexyl ester. To a stirring solution of alcohol **1.94** (300 mg, 0.359 mmol, 1.0 equiv), DMAP (4.0 mg, 0.035 mmol, 0.1 equiv) in CH_2Cl_2 (3.6 mL) in a 10 mL round bottom flask at rt, was added a premixed solution of triethylamine (150 µL, 0.718 mmol, 3.0 equiv) and acetic anhydride (67 µL, 0.718 mmol, 2.0 equiv) dropwise via syringe. TLC analysis after 24 h indicated completion of reaction. The reaction was diluted with 20% EtOAc/hexanes (50 mL), washed with sat. $NaHCO_3$ solution (2 × 25 mL), brine (2 × 25 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the crude product as a pale yellow oil. Purification was accomplished by flash column chromatography eluting with 5% EtOAc/hexanes providing acylated product (307 mg, 98% yield) as a colorless oil: R_f

= 0.40 (10% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.71 – 7.67 (m, 4H), 7.44 – 7.35 (m, 6H), 7.20– 7.19 (m, 2H), 6.84 – 6.82 (m, 2H), 5.27 (d, *J* = 10.25 Hz, 1H), 4.89 (s, 1H), 4.82 (s, 1H), 4.24 (quin, *J*=5.8 Hz, 1H), 4.20 (ABq, *J* = 10.2 Hz, Δν = 29.0 Hz, 2H), 3.80 (s, 3H), 3.65 (t, *J* = 7.3 Hz, 2H), 3.24 – 3.19 (m, 1H), 2.66 (dd, *J* = 14.6, 6.3 Hz, 1H), 2.56 (dd, *J* = 14.1, 5.8 Hz, 1H), 2.29 (ddd, *J*= 15.0, 7.3, 7.3 Hz, 1H), 2.23 (ddd, *J*= 15.0, 6.2, 6.2 Hz, 1H), 1.99 (s, 3H), 1.97 – 1.90 (m, 1H), 1.52 – 1.46 (m, 1H), 1.43 (s, 9H), 1.32 – 1.17 (m, 2H), 1.04 (s, 9H), 0.95 (s, 3H), 0.93 (s, 3H), 0.89 (s, 9H), 0.03 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 197.7, 170.8, 159.1, 150.8, 136.1, 136.0, 133.9, 131.0, 129.9, 129.9, 129.6, 127.8, 113.7, 111.1, 74.6, 73.4, 71.3, 68.7, 63.3, 55.4, 52.3, 48.2, 43.4, 42.8, 35.8, 34.7, 29.9, 27.1, 26.1, 24.1, 22.8, 21.6, 21.3, 19.5, 18.5, -5.0, -5.0.



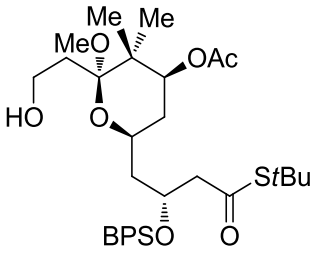
Preparation of 1-[4-(*tert*-butyl-dimethyl-silanyloxy)-1,1-dimethyl-2 methylene-butyl]-5-(*tert*-butyl-diphenyl-silanyloxy)-6-*tert*-butylsulfanylcarbonyl-3- hydroxy-hexyl ester (1.95). To a stirring solution of PMB ether mentioned above (881 mg, 1.005 mmol, 1.0 equiv) in CH_2Cl_2 (7 mL), and pH 7.0 phosphate buffer (3 mL) in a 25 mL round bottom flask, at rt, was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (456 mg, 2.01 mmol, 2.0 equiv) in one portion. After 2 h the reaction mixture diluted with 20% EtOAc/hexanes (100 mL), and filtered over a plug of Celite®, Florsil®, and Na_2SO_4 (2 cm each). The filtrate was concentrated under reduced pressure to give the crude product as a pale yellow oil. Purification was accomplished by flash column chromatography eluting with 5% EtOAc/hexanes providing alcohol **1.95** (621 mg, 94% yield) as a colorless oil: R_f = 0.654 (25% EtOAc/hexanes); 500 MHz ^{1}H

NMR ($CDCl_3$) δ 7.70 – 7.67 (m, 4H), 7.45 – 7.38 (m, 6H), 5.06(dd, *J* = 11.4, 1.5 Hz, 1H), 4.93(s, 1H), 4.85 (s, 1H), 4.41 – 4.36 (m, 1H), 3.74 (dddd, *J* = 17.9, 9.9, 7.0, 7.0 Hz, 2H), 3.47 (t, *J* = 7.8 Hz, 2H), 2.63 – 2.61 (m, 3H), 2.24 (t, *J* = 7.3 Hz, 2H), 1.93 (s, 3H), 1.60 (ddd, *J* = 14.3, 9.2, 4.0 Hz, 1H), 1.55 (ddd, *J* = 14.3, 8.1, 2.6 Hz, 1H), 1.45 – 1.23 (m, 2H), 1.40 (s, 9H), 1.03 (s, 9H), 1.01(s, 3H), 1.01(s, 3H), 0.90 (s, 9H), 0.06 (s, 6H), 125 MHz ^{13}C NMR ($CDCl_3$) δ 197.7, 172.2, 150.6, 136.1, 136.0, 133.8, 133.8, 129.9, 129.9, 127.9, 127.8, 111.3, 75.3, 68.5, 63.8, 63.3, 52.5, 48.1, 44.4, 42.9, 38.1, 34.7, 29.9, 27.1, 26.1, 24.0, 21.0, 19.6, 18.5, -5.0, -5.0.



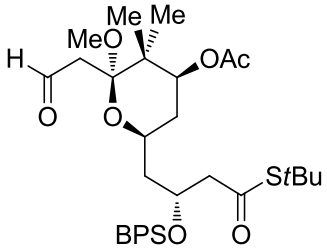
Preparation of 2-[2-(*tert*-butyl-dimethyl-silanyloxy)-ethyl]-6-[2-(*tert*-butyl-diphenyl-silanyloxy)-3-*tert*-butylsulfanylcarbonyl-propyl]-2-3,3-dimethyl-tetrahydro-pyran-4-yl ester (1.96). To a stirring solution of alkene **1.95** (571 mg, 0.754 mmol, 1.0 equiv) in CH_2Cl_2 (15.0 mL) in a 25 mL round bottom flask, at -78° C, was bubbled a steady stream of ozone for 5 min. The solution color changed to light blue. The ozone stream was stopped and the excess O_3 was removed by passing a stream of N_2. The reaction mixture was concentrated under reduced pressure, dimethyl methyl sulfide (20 mL) was added and the resulting solution was stirred for 12 h. The solution was concentrated under reduced pressure to give the crude product. Purification was accomplished by flash column chromatography eluting with 5% EtOAc/hexanes providing cyclic hemiketal **1.96** (486 mg, 85% yield) as a colorless oil. R_f = 0.55 (20% EtOAc/hexanes); 500 MHz ^1H NMR ($CDCl_3$) δ 7.72 – 7.68 (m, 4H), 7.43

– 7.36 (m, 6H), 5.22 (s, 1H), 5.06 (dd, *J* = 11.7, 4.8 Hz 1H), 4.30 (dddd, *J* = 10.2, 5.3, 5.3, 5.3, 1H), 4.17 (ddd, *J* = 12.1, 10.3, 1.8 Hz, 1H), 3.80 (dddd, *J* = 11.7, 7.3, 4.4, 2.9 Hz, 1H), 3.73 (ddd, *J* = 10.3, 4.0, 2.9, 1H), 2.69 (d, *J* = 5.8 Hz, 2H), 2.01 (s, 3H), 1.91–1.85 (m, 1H), 1.72 (td, *J* = 14.1, 7.8 Hz, 1H), 1.58 – 1.41 (m, 3H), 1.44 (s, 9H), 1.36 (ddd, *J* = 12.1, 4.8, 2.9 Hz, 1H), 1.03 (s, 9H), 0.91(s, 3H), 0.89 (s, 9H), 0.83 (s, 3H), 0.09 (s, 3H), 0.09 (s, 3H), 125 MHz ^{13}C NMR ($CDCl_3$) δ 197.9, 170.5, 136.1, 136.0, 134.6, 129.8, 129.7, 127.7, 127.7, 102.2, 73.4, 69.3, 64.3, 60.2, 52.4, 48.0, 43.8, 41.3, 34.0, 33.5, 30.0, 27.1, 26.0, 21.3, 21.1, 19.6, 18.2, 16.8, -5.2, -5.3.



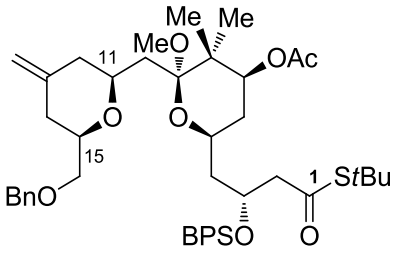
Preparation of acetic acid 6-[2-(*tert*-butyl-diphenyl-silanyloxy)-3-*tert*-butylsulfanylcarbonyl-propyl]-2-(2-hydroxy-ethyl)-2methoxy-3,3-dimethyl-tetrahydro-pyran-4-yl ester). To a stirring solution of TBS ether **1.96** (0.42 g, 0.553 mmol, 1.0 equiv) and MeOH (5.5 mL) in a 10 mL round bottom flask, at rt, was added (±)-camphor-10-sulfonic acid (32 mg, 0.138 mmol, 0.25 equiv) in one portion. The reaction mixture was allowed to proceed for 1.5 h, after which time TLC analysis indicated completion of reaction. The reaction mixture was quenched by pouring the reaction mixture into a mixture of saturated aqueous $NaHCO_3$ solution (50 mL) and 50% EtOAc/hexanes (100 mL). The resulting layers were separated and the aqueous layer was extracted with 50% EtOAc/hexanes (2 × 25 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography eluting with

15% EtOAc/hexanes providing cyclic methyl ketal (312 mg, 86%) as a colorless oil: R_f = 0.18 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.73 – 7.64 (m, 4H), 7.46 – 7.36 (m, 6H), 5.05(dd, *J* = 11.7, 4.7 Hz, 1H), 4.27 (q, *J* = 5.9, 1H), 3.68 – 3.58 (m, 2H), 3.47 (dddd, *J* = 11.7, 7.3, 4.0, 4.0 Hz, 1H), 2.97 (s, 3H), 2.72 (dd, *J* = 14.2, 5.8 Hz, 1H), 2.62 (dd, *J* = 14.6, 6.2 Hz, 1H), 2.32 (t, *J* = 6.2 Hz, 1H), 2.06 – 1.96 (m, 1H), 2.02 (s, 3H), 1.83 – 1.72 (m, 2H), 1.53 – 1.41 (m 2H), 1.43 (s, 9H), 1.13 –1.10 (m, 1H), 1.03 (s, 9H), 0.90 (s, 3H), 0.82 (s, 3H), 125 MHz ^{13}C NMR ($CDCl_3$) δ 198.0, 170.7, 136.1, 136.0, 134.0, 133.9, 130.0, 130.0, 127.9, 127.9, 104.9, 73.4, 68.8, 66.0, 59.7, 52.6, 48.6, 48.4, , 43.9, 42.0, 34.7, 32.9, 29.9, 27.1, 21.4, 20.5, 19.5, 17.3.



Preparation of acetic acid 6-[2-(*tert*-butyl-diphenyl-silanyloxy)-3-*tert*-butylsulfanylcarbonyl-propyl]-2-methoxy-3,3-dimethyl-2-(2-oxo-ethyl)-tetrahydro-pyran-4-yl ester (1.69). To a stirring solution of alcohol from above reaction (183 mg, 0.277 mmol**,** 1.0 equiv) in CH_2Cl_2 (9.2 mL) in a 25 mL round bottom flask under an atmosphere of N_2, at -5 °C, was added freshly distilled *N*,*N*-diisopropylethylamine (634 µL, 1.939 mmol, 7 equiv), dropwise via syringe. After 10 min at -5 °C, dimethyl sulfoxide (160 µL, 2.77 mmol, 10.0 equiv) was added to the reaction mixture via syringe and the solution was allowed to stir for an additional 10 min. Sulfur trioxide pyridine complex (176 mg, 1.18 mmol, 4.0 equiv) was then added in one portion. The reaction was allowed to proceed for 1 h at -5 °C, and the reaction was diluted with 20% EtOAc/hexanes (100 mL) and quenched by pouring into saturated

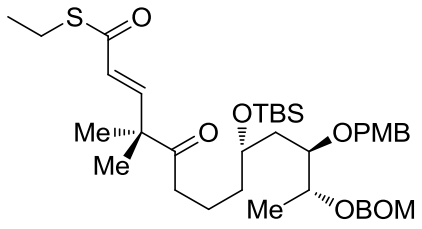
aqueous $NaHCO_3$ solution (50 ml). The layers were separated and the aqueous phase was extracted with 20% EtOAc/hexanes (2× 25 mL). The combined organic layers were washed with brine (2 × 25 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product. Purification was accomplished by flash column chromatography eluting with 10% EtOAc/hexanes providing aldehyde **1.69** (170 mg, 94% yield) as colorless oil: R_f = 0.55 (25% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.66 (t, *J* = 2.9 Hz, 1H), 7.70 – 7.65 (m, 4H), 7.46 – 7.36 (m, 6H), 5.02(dd, *J* = 11.7, 4.8 Hz, 1H), 4.27 (q, *J* = 6.3, 1H), 3.68 – 3.58 (m, 1H), 3.47 (dddd, *J* = 11.7, 7.8, 4.3, 4.3 Hz, 1H), 2.97 (s, 3H), 2.72 (dd, *J* = 14.1, 6.3 Hz, 1H), 2.62 (dd, *J* = 14.1, 6.3 Hz, 1H), 2.32 (t, *J* = 3.4 Hz, 1H), 2.06 – 1.96 (m, 1H), 2.02 (s, 3H), 1.83 – 1.72 (m, 2H), 1.53 – 1.41 (m 2H), 1.43 (s, 9H), 1.13 –1.10 (m, 1H), 1.03 (s, 9H), 0.90(s, 3H), 0.82 (s, 3H), 125 MHz ^{13}C NMR ($CDCl_3$) δ 198.0, 170.7, 136.1, 136.0, 134.0, 133.9, 130.0, 130.0, 127.9, 127.9, 104.9, 73.4, 68.8, 66.0, 59.7, 52.6, 48.6, 48.4, , 43.9, 42.0, 34.7, 32.9, 29.9, 27.1, 21.4, 20.5, 19.5, 17.3.



Preparation of acetic acid 2-(6-benzyloxymethyl-4-methylene-tetrahydro-pyran-2-ylmethyl)-6-[2-(*tert*-butyl-diphenyl-silanyloxy)-3-*tert*-butyl sulfanylcarbonyl-propyl]-2-methoxy-3, 3-dimethyl-tetrahydro-pyran-4-yl ester (1.100). To a stirring solution of aldehyde **1.69** (19.0 mg, 0.029 mmol, 1.0 equiv) in Et_2O (0.414 mL) in a 5 ml reaction vial, under N_2 at –78 °C, was added hydroxyl allylsilane **1.99** (8.8 mg, 0.0319 mmol, 1.1 equiv, in 0.05 ml Et_2O)

via syringe and the mixture was stirred for 10 min. A solution of TMSOTf in Et_2O (0.034 ml of 1.014 M, 0.0348 mmol, 1.2 equiv) was then added dropwise via syringe. The reaction was allowed to proceed for 5 h at –78 °C. The reaction mixture was then quenched at –78 °C by the addition of Hunig's base (0.05 ml) and stirred for 10 min. The reaction was warmed to 0 °C and again quenched by the addition of saturated aqueous $NaHCO_3$ solution (3 mL). The reaction was diluted with 25 ml of 25% EtOAc in hexanes. The layers were separated and the aqueous layer was extracted with 25% EtOAc in hexanes (2 × 10 mL). The combined organic layers were washed with brine (2 × 10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 1 × 25 cm column, eluting with 5% EtOAc/hexanes, collecting 3 mL fractions. The product containing fractions (30-41) were combined and concentrated under reduced pressure to give **1.100** (21 mg, 85% yield) as a colorless viscous oil; R_f = 0.48 (20% EtOAc/hexanes); $[\alpha]^{20}_D$ = + 21 (*c* = 1.15, $CHCl_3$); 500 MHz ^{1}HNMR ($CDCl_3$) δ 7.70-7.66 (m, 4H), 7.43-7.34 (m, 11H), 4.97 (dd, *J* = 11.7, 5.12 Hz, 1H), 4.72 (s, 2H), 4.59 (s, 2H), 4.26 (dddd, *J* = 10.98, 3.84, 3.84, 3.84 Hz, 1H), 3.59-3.42 (m, 4H), 3.19 (dddd, *J* = 11.23, 7.29, 3.84, 3.81, 1H), 2.95 (s, 3H), 2.75(dd, *J* = 15.05, 4.76 Hz, 1H), 2.71 (dd, *J* = 14.83, 7.12 Hz, 1H), 2.27-2.18 (m, 2H), 2.0 (s, 3H), 2.04-1.88 (m, 4H), 1.69-1.64 (m, 2H), 1.45 (s, 9H), 1.32-1.26 (m, 2H), 1.03 (s, 9H), 0.90 (s, 3H), 0.84 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 198.2, 170.7, 144.5, 138.7, 136.1, 136.0, 134.4, 133.6, 129.9, 129.8, 128.5, 127.8, 127.78, 127.72, 127.6, 109.1, 104.0, 77.5, 75.0, 73.8, 73.5, 73.4, 69.5, 66.0, 53.2, 48.3, 48.1, 43.7, 42.3, 42.0, 39.2, 37.2, 32.8, 30.0, 27.1, 21.4, 20.7, 19.5, 16.8; IR (thin film) 2958, 2933, 2895, 2859, 1742, 1682, 1455, 1428, 1365, 1245, 1111, 892, 822, 612, 703 cm^{-1} ; HRMS (EI+) calcd

for $C_{49}H_{68}O_8SSi$ 844.4404, found 844.4432. Verification of C_{11}-C_{15} stereochemistry: Following NOE interaction proved the expected stereochemistry about the A and the B-ring.

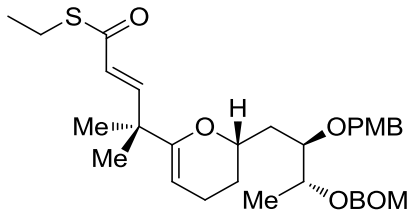


Preparation of (9*S*,11*R*,12*R*,*E*)-*S*-ethyl 12-((benzyloxy)methoxy)-9-((*tert*-butyldimethylsilyl)oxy)-11-((4-methoxybenzyl)oxy)-4,4-dimethyl-5-oxotridec-2-enethioate (1.111): To a stirring solution of alkene **1.77** (500 mg, 0.81 mmol, 1.0 equiv.) in 20:1 EtOAc/ MeOH (16 mL), in a 50 mL rb flask, at -78 °C was added $NaHCO_3$ (320 mg, 8.15 mmol, 10.0 equiv). A steady stream of O_3 was bubbled through the reaction mixture until a light blue color developed. TLC showed the completion of the reaction. The excess O_3 was removed by bubbling O_2 through the mixture until the light blue color faded. A solution of PPh_3 (320 mg, 1.2 mmol, 1.5 equiv.) in CH_2Cl_2 (2 mL) was added to the reaction mixture, and the reaction mixture was slowly warmed to rt and stirred for 12 h. The solids were removed by filtration, and the solution was concentrated under reduced pressure. The resulting yellow oil was taken-up in 10% Et_2O/ pentane (20 mL) in a 50 mL rb flask, and placed in a -20 °C freezer for 6 h during which the triphenylphosphine oxide precipitate was formed. The white precipitate was removed by filtration, and rinsed with 10 mL of ice cold 1% Et_2O/ pentane. The solvent was removed under reduced pressure to yield crude aldehyde as a light yellow oil, which was taken on to the next step without further purification.

To a stirring solution of S-ethyl 2-(diethoxyphosphoryl)ethanethioate (430 mg, 1.7 mmol, 2.2 equiv) in THF (3 mL) in a 10 mL rb flask at 0 °C, was added NaH (43 mg,

1.7 mmol, 2.2 equiv.) slowly over 10 min. The reaction mixture was stirred at 0 °C for an additional 30 min, and a solution of crude aldehyde in THF (1 mL) was added dropwise to the reaction mixture slowly via cannula and rinsed with THF (0.5 mL). Stirring continued at 0 °C for an addition 2 h after which the reaction was quenched by adding EtOAc (15mL) and a saturated aqueous NH_4Cl solution (20 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic phases were washed with brine (25 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification was accomplished using flash column chromatography eluting with 10% EtOAc/ hexanes. The product containing fractions were combined and concentrated under reduced pressure to provide thiolester **1.111** (481 g, 84% over 2 steps) as a clear colorless oil: R_f = 0.51 (20% EtOAc/ hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.36-7.23 (m, 7H), 6.95 (d, *J* = 15.9 Hz, 1H), 6.88-6.85 (m, 2H), 6.13 (d, *J* = 15.9 Hz, 1H), 4.81 (d, *J* = 7.3 Hz, 1H), 4.79 (d, *J* = 7.3 Hz, 1H), 4.66 (d, *J* = 12.0 Hz, 1H), 4.60 (d, *J* = 11.6 Hz, 1H), 4.59 (d, *J* = 10.7 Hz, 1H), 4.44 (d, *J* = 10.7 Hz, 1H), 4.51 (qd, *J* = 6.4, 4.7 Hz, 1H), 3.89 (m, 1H), 3.80 (s, 3H), 3.64 (ddd, *J* = 9.4, 4.5, 2.3 Hz, 1H), 2.97 (q, *J* = 7.4 Hz, 2H), 2.41(m, 2H), 1.68 (ddd, *J* = 15.1, 8.5, 2.6 Hz, 1H), 1.60-1.53 (m, 3H), 1.45-1.40 (m, 2H), 1.29 (t, *J* = 7.5 Hz, 3H), 1.27 (s, 6H), 1.18 (d, *J* = 6.4 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 3H), 0.04 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 210.5, 189.9, 159.2, 147.2, 138.1, 131.0, 129.2, 128.5(x2), 127.9, 127.7, 127.6, 113.8, 93.3, 78.1, 72.9, 72.0, 69.5, 69.4, 55.3, 50.5, 38.5, 37.7, 37.3, 26.1, 23.5, 23.4, 19.1, 18.2, 15.1, 14.8, -3.6, -4.2; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 55.3, 26.0, 23.5, 15.1, 14.8, -3.6, -4.2; CH_2 δ 93.2, 71.9, 69.4, 38.5, 37.7, 37.3, 23.4, 19.1; CH_1 δ 147.2, 129.2, 128.5,

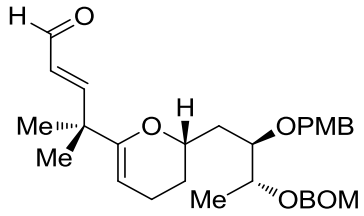
127.9, 127.7, 127.6, 113.8, 78.1, 72.8, 69.5; CH_0 δ 210.5, 189.9, 159.2, 138.1, 131.0, 50.5, 18.2.



Preparation of (*E*)-*S*-ethyl 4-((*S*)-2-((2*R*,3*R*)-3-((benzyloxy) methoxy)-2-((4-methoxybenzyl)oxy)butyl)-3,4-dihydro-2H-pyran-6-yl)-4-methylpent-2-enethioate (1.112): To a stirring solution of thiolester **1.111** (102 mg, 0.14 mmol, 1.0 equiv.) in 20:1 CH_3CN/ H_2O (3 mL), in a plastic bottle at 0 °C, was added pyridine (480 µL, 0.3 M) and aqueous HF solution (48%, 0.1 mL). The solution was stirred at 0 °C for 30 min and warmed to rt after which it was brought to rt. After 30 min at rt, an additional 0.1 mL of aqueous HF solution (48%) was added every h until TLC analysis indicated complete consumption of the starting material. The reaction was quenched by slowly pipetting the reaction mixture into a mixture of saturated aqueous $NaHCO_3$ solution (25 mL) and EtOAc (25 mL). The phases were separated and the aqueous phase was extracted three times with EtOAc (3 x 25 mL). The combined organic phases were washed saturated aqueous $CuSO_4$ solution (2 x 25 mL), and with brine (2 x 25 mL). The combined organic phases were dried with Na_2SO_4, filtered and concentrated to provide the crude intermediate alcohol as clear light yellow oil. This crude material was carried on to the next step without further purification.

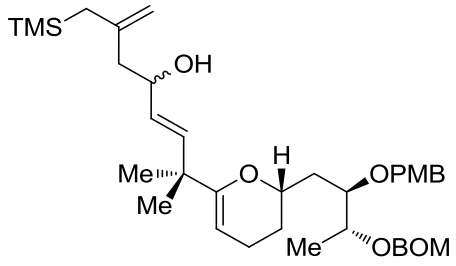
To a stirring solution of the previously described intermediate crude alcohol in benzene (3 mL) in a 15mL rb flask equipped with a condenser and Dean-Stark trap, was added CSA (2 mg, 0.007 mmol, 0.05 equiv.). The solution was heated to reflux for 1 h and allowed to cool to room temperature. The reaction mixture was quenched with

pyridine (0.1 mL), and the solvent was removed under reduced pressure. This material was immediately purified using flash column chromatography on a 1 x 10 cm silica gel column, eluting with 5% EtOAc/ hexanes, collecting 5 mL fractions. The product containing fractions (15-42) were combined and concentrated under reduced pressure to yield pure dihydropyran **1.112** (73 mg, 88% over 2 steps) as a clear colorless oil: R_f = 0.48 (20% EtOAc/ hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.36-7.35 (m, 4H), 7.33-7.27 (m, 1H), 7.24 (d, *J* = 8.6 Hz, 2H), 6.97 (d, *J* = 15.9 Hz, 1H), 6.85 (d, *J* = 8.6 Hz, 1H), 6.08 (d, *J* = 15.9 Hz, 1H), 4.83 (d, *J* = 6.9 Hz, 1H), 4.81 (d, *J* = 6.9 Hz, 1H), 4.66-4.58 (m, 4H), 4.48 (d, *J* = 11.1 Hz, 1H), 4.02-3.93 (m, 2H), 3.84-3.78 (m, 4H), 2.88 (q, *J* = 7.4 Hz, 2H), 2.12-2.04 (m, 1H), 2.01-1.95 (m, 1H), 1.81-1.74 (m, 2H), 1.63-1.56 (m, 1H), 1.54-1.46 (m, 1H), 1.23-1.19 (m, 12H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 190.5, 159.3, 157.0, 151.9, 138.1, 131.0, 129.6, 128.5 (x2), 128.0, 127.8, 125.7, 113.9, 94.5, 93.7, 77.7, 73.8, 73.6, 71.9, 69.5, 55.4, 41.4, 36.2, 28.2, 25.2, 25.1, 23.2, 20.5, 15.5, 14.9; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 55.4, 25.2, 25.1, 15.5, 14.9; CH_2 δ 93.5, 73.6, 69.5, 36.2, 28.1, 23.2, 20.5; CH δ 151.9, 129.6, 128.5, 128.0, 127.8, 125.7, 113.9, 94.5, 77.7, 73.8, 71.9; CH_0 δ 190.5, 159.3, 157.0, 138.1, 131.0, 41.4.



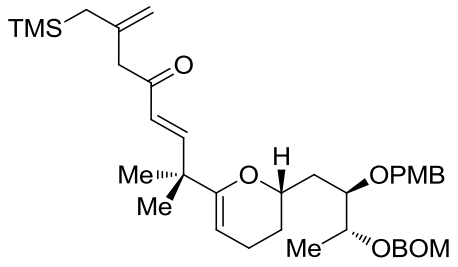
Preparation of (*E*)-4-((*S*)-2-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-3,4-dihydro-2H-pyran-6-yl)-4-methylpent-2-enal (1.76): To a stirring solution of thioester **1.112** (457 mg, 0.803 mmol, 1 equiv.) in CH_2Cl_2 (8 mL) at - 78 ^oC was added a solution of DIBAL-H in CH_2Cl_2 (5.5 mL of 1.0 M, 1.1 mmol, 3 equiv.) dropwise over a period of 30 min. This

mixture was stirred at -78 ^{o}C for 1.5 h then EtOAc (2 mL) was added dropwise over 10 min. The solution was stirred for 15 min, and then quenched by the addition of saturated aqueous Rochelle salt (5 mL) dropwise. The cold bath was removed, and the mixture was stirred vigorously for 3 h at ambient temperature. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (2 x 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified *via* flash column chromatography, eluting with 10% EtOAc/hexanes collecting 4 mL fractions. The product containing fractions (35-80) were collected and concentrated under reduced pressure to provide α,β-unsaturated aldehyde **1.76** (327 mg, 80%) as a colorless oil: R_f = 0.54 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.47 (d, *J* = 7.8 Hz, 1H), 7.37 – 7.23 (m, 8H), 6.87 – 6.83 (m, 4H), 6.10 (dd, *J* = 16.1, 7.81 Hz, 1H), 4.81 (ABq, Δν = 10.7 Hz, 2H), 4.62 – 4.60 (m, 4H), 4.46 (d, *J* = 10.7 Hz, 1H), 4.05 – 3.94 (m, 2H), 3.80 – 3.75 (m, 1H), 3.79 (s, 3H), 2.13 - 2.06 (m,1H), 2.03 – 1.97 (m, 1H), 1.83 – 1.76 (m, 2H), 1.64 – 1.58 (m, 1H), 1.55 – 1.47 (m, 1H), 1.25 (d, *J* = 3.4 Hz, 6H), 1.20 (d, *J* = 6.3 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 194.5, 165.5, 159.3, 156.6, 138.0, 130.8, 129.7, 129.4, 128.5, 127.9, 127.7 113.9, 94.7, 93.5, 77.5, 73.5, 73.2, 72.0, 69.5, 55.3, 42.0, 35.1, 28.0, 25.2, 25.0, 20.4, 15.4; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 55.3, 25.2, 25.1, 15.4; CH_2 δ 93.4, 73.2, 69.5, 35.9, 28.0, 20.4; CH δ 194.5, 165.5, 129.7, 129.4, 128.5, 127.9, 113.9, 94.7, 77.5, 73.5, 72.0; CH_0 δ 165.5, 159.3, 156.6, 138.0, 130.8, 42.0.



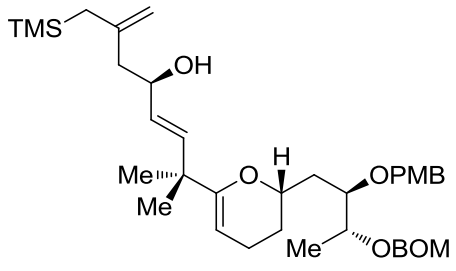
Preparation of (*E*)-7-((*S*)-2-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-3,4-dihydro-2H-pyran-6-yl)-7-methyl-2-((trimethylsilyl)methyl) octa-1,5-dien-4-ol (1.113): To a solution of aldehyde **1.76** (625 mg, 1.22 mmol, 1 equiv.) in toluene (12 mL) in a 25 mL rb flask was added a trimethyl(2-((tributylstannyl)methyl)allyl)silane via syringe. The mixture was heated to reflux at 120 ^{o}C for 24 h in which TLC showed the completion of reaction. The reaction mixture was allowed to cool to rt and the solvent was removed under reduced pressure. Purification was accomplished using flash column chromatography with a 3 x 18 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 6 mL fractions. The product containing fractions (51-115) were combined and concentrated under reduced pressure to provide alcohol **1.113** (702 mg, 90%) as 1:1 mixture of diastereomers as colorless oil. R_f = 0.57 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +21.2 (*c* = 1.03, EtOAc); 500 MHz ^{1}H NMR (C_6D_6) δ 7.38-7.08 (m, 7H), 6.82-6.80 (m, 2H), 6.04 (dd, *J* = 15.6, 6.3 Hz, 1H), 5.67 (dd, *J* = 15.6, 5.8 Hz, 1H), 4.83-4.70 (m, 4H), 4.67-4.6 (m, 4H), 4.29-4.4.05 (m, 4H), 3.30 (s, 3H), 2.59-2.50 (m, 2H), 2.29-2.15 (m, 2H), 2.02-1.83 (m, 5H), 1.76-1.71 (m, 1H), 1.64-1.38 (m, 5H), 1.33 (dd, *J* = 8.7, 2.4 Hz, 6H), 1.23 (dd, *J* = 6.3 Hz, 4H), 0.00 (s, 9H); 125 MHz ^{13}C NMR (C_6D_6) δ 160.0 (x2), 159.7, 159.6, 144.9, 139.2, 138.8, 138.7, 132.1 (x2), 130.4 (x2), 129.9, 129.8, 128.9, 128.0, 114.4, 111.0, 110.9, 93.8 (x2), 78.2, 78.1, 74.0, 73.9, 73.6, 73.5, 72.4 (2), 71.0, 70.8, 69.8, 69.7, 55.1, 47.8, 41.1 (x2), 36.3 (x2), 28.8 (x2), 27.3 (x2), 26.7 (x2), 26.5, 21.0 (x2), 15.5 (x2),

-0.9; 125 MHz DEPT ^{13}C NMR (C_6D_6) CH_3 δ 55.1, 27.7, 26.5, 15.5, 0.93; CH_2 δ 111.0, 93.8, 73.5, 69.7, 47.8, 47.7, 36.3, 36.2, 28.8, 27.3, 21.0; CH_1 δ 138.8 (x2), 130.4, 129.9, 129.8, 128.0, 114.4, 111.0, 93.8, 78.1, 74.0, 73.9, 72.4, 71.0, 70.8, 69.7; IR (neat) 2959, 2858, 1738, 1606, 1513, 1461, 1427, 1365, 1247, 1160, 1077, 822, 740, 703 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{38}H_{56}NaO_6Si$ (M+Na): 659.3744, found: 659.3746.



Preparation of (*E*)-7-((*S*)-2-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-3,4-dihydro-2H-pyran-6-yl)-7-methyl-2-((trimethylsilyl) methyl)octa-1,5-dien-4-one (1.114): To a stirring solution of alcohol **1.113** (700 mg, 1.09 mmol**,** 1.0 equiv) in CH_2Cl_2 (11 mL) in a 25 mL rb rlask at -15 °C, was added freshly distilled *N*,*N*-diisopropylethylamine (1.3 mL, 7.6 mmol, 7.0 equiv), dropwise via syringe. After 10 min at -15 °C, dimethyl sulfoxide (780 µL, 10.9 mmol, 10.0 equiv) was added to the reaction mixture via syringe and the solution was allowed to stir for an additional 10 min. Sulfur trioxide pyridine complex (700 mg, 4.3 mmol, 4.0 equiv) was then added in one portion. The reaction mixture was allowed to proceed for 1 h at -15 °C, after which time TLC analysis indicated complete consumption of starting material. The reaction mixture was diluted with EtOAc (10 mL), quenched by addition of saturated aqueous $NaHCO_3$ solution (20 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2x20 mL). The combined organic layers were washed with brine (2 × 20 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column

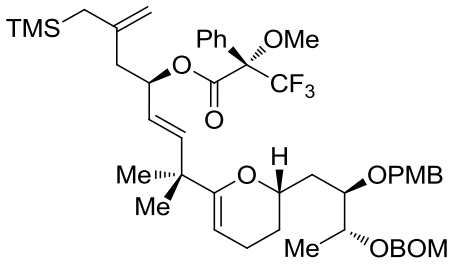
chromatography on a 3 × 13 cm column, eluting with 10% EtOAc/hexanes, collecting 6 mL fractions. The product containing fractions (20-55) were combined and concentrated under reduced pressure to give the ketone **1.114** (658 mg, 94% yield) as colorless oil: R_f = 0.53 (20% EtOAc/hexanes); $[\alpha]_D^{20}$ = +44 (*c* = 1.0, EtOAc); 500 MHz ^{1}H NMR (C_6D_6) δ 7.39-7.09 (m, 7H), 6.83-6.81 (m, 2H), 6.34 (d, *J* = 15.6 Hz, 1H), 4.81-4.75 (m, 4H), 4.69 (d, *J* = 10.7 Hz, 2H), 4.63 (d, *J* = 2.9 Hz, 1H), 4.59 (d, *J* = 11.2 Hz, 2H), 4.53 (t, *J* = 3.9 Hz, 1H), 4.14-4.00 (m, 3H), 3.30 (s, 3H), 3.11 (s, 2H), 1.98-1.89 (m, 1H), 1.86 (ddd, *J* = 9.2, 6.8, 2.4 Hz, 1H), 1.81-1.75 (m, 1H), 1.61-1.56 (m, 3H), 1.51-1.47 (m, 1H), 1.41-1.32 (m, 1H), 1.23 (s, 3H), 1.22 (d, *J* = 4.3 Hz, 3H), 1.19 (s, 3H), -0.00 (s, 9H); 125 MHz ^{13}C NMR (C_6D_6) δ 197.0, 160.4, 157.9, 154.1, 142.3, 139.2, 132.0, 129.8, 128.9, 128.3, 128.0, 126.4, 114.4, 112.4, 94.9, 93.8, 77.9, 73.6, 73.5, 72.7, 69.7, 55.1, 41.8, 36.3, 28.7, 27.6, 25.6 (x2), 21.0, 15.4, -0.9; 125 MHz DEPT ^{13}C NMR (C_6D_6) CH_3 δ 55.1, 25.6 (x2), 15.4, -0.9; CH_2 δ 112.4, 93.8, 73.5, 69.7, 51.5, 36.3, 28.9, 27.2, 21.0; CH_1 δ 154.1, 129.8, 128.9, 128.7, 128.3, 126.4, 114.4, 94.9, 78.0, 73.7, 72.7; CH_0 δ 197.0, 160.0, 157.9, 142.3, 139.2, 132.0, 128.0; IR (neat) 3071, 2956, 2858, 1742, 1682, 1629, 1461, 1427, 1365, 1246, 1111, 1027, 978, 853, 756, 703, 612 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{38}H_{58}NO_6Si$ (M+NH_4): 652.9558, found: 652.4033.



Preparation of (*R*,*E*)-7-((*S*)-2-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-3,4-dihydro-2H-pyran-6-yl)-7-methyl-2-((trimethylsilyl)methyl) octa-1,5-dien-4-ol (1.68): To a stirring solution of

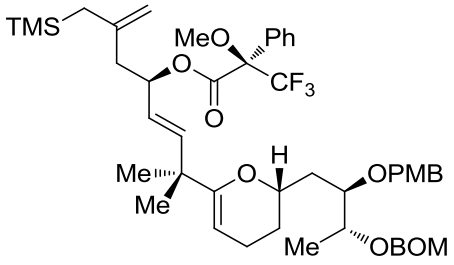
BH_3·DMS (1M in CH_2Cl_2, 1 mL, 1.03 mmol, 1 equiv) in 10 mL of THF in a flame dried 50 mL rb flask at 0 ºC was added *S*-CBS (1 M in toluene, 0.2 mL, 2.07 mmol, 2 equiv) dropwise via syringe. The solution was stirred at 0 ºC for 15 min and was then cooled to -42 ºC. To this solution was added a solution of ketone **1.114** in 1 mL of toluene dropwise via syringe down the side of the flask. The reaction was stirred at -42 ºC for 3 h after which it was quenched by slow addition of MeOH. The reaction was allowed to come to rt and the solvent was evaporated under reduced pressure. Purification was accomplished using flash column chromatography with a 3 x 15 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 6 ml fractions. The fraction 24-37 provided the starting material (98 mg) and the product containing fractions (38-135) were combined and concentrated under reduced pressure to provide the alcohol **1.68** (485 mg, 74%) as a clear colorless oil: R_f = 0.47 (30% EtOAc/hexanes); $[\alpha]_D^{20} = +26$ (*c* = 1.0, $CHCl_3$); 500 MHz ^{1}H NMR (C_6D_6) δ 7.39-7.10 (m, 7H), 6.82-6.80 (m, 2H), 6.05 (d, *J* = 16.4 Hz, 1H), 5.67 (dd, *J* = 15.6, 6.3 Hz, 1H), 4.82 (d, *J* = 6.8 Hz, 2H), 4.77 (d, *J* = 6.8 Hz, 1H), 4.74 (s, 1H), 4.73 (d, *J* = 11.2 Hz, 1H), 4.68-4.67 (m, 3H), 4.62 (d, *J* = 12.2 Hz, 1H), 4.29 (dd, *J* = 12.6, 6.5 Hz, 1H), 4.22-4.18 (m, 1H), 4.12 (td, *J* = 4.9, 1.9 Hz, 1H), 4.10-4.05 (m, 1H), 3.29 (s, 3H), 2.29-2.21 (m, 2H), 1.99 (ddd, *J* = 13.6, 10.2, 1.4 Hz, 1H), 1.94 (ddd, *J* = 9.7, 6.8, 2.4 Hz, 1H), 1.89-1.83 (m, 1H), 1.77 (s, 1H), 1.60 (ddd, *J* = 13.1, 10.7, 2.4 Hz, 1H), 1.56-1.53 (m, 1H), 1.50 (d, *J* = 2.9 Hz, 3H), 1.47-1.37 (m, 2H), 1.34 (s, 3H), 1.33 (s, 3H), 1.23 (d, *J* = 6.3 Hz, 3H), -0.00 (s, 9H); 125 MHz ^{13}C NMR (C_6D_6) δ 160.0, 159.6, 144.9, 139.1, 138.8, 132.0, 130.4, 129.9, 128.9, 128.3, 128.0, 114.4, 110.9, 93.8 (x2), 78.1, 74.0, 73.6, 72.4, 71.0, 69.7, 55.41, 47.7, 41.1, 36.2, 28.8, 27.3, 26.7, 26.5, 21.0, 15.5, -0.9; 125 MHz DEPT ^{13}C NMR (C_6D_6) CH_3 δ 55.1, 26.7,

26.5, 15.5, -0.9; CH_2 δ 110.9, 93.8, 73.6, 69.7, 47.7, 36.2, 28.8, 27.3, 21.0; CH_1 δ 138.8, 130.4, 129.9, 128.9, 128.3, 128.0, 114.4, 93.8, 78.1, 74.0, 72.4, 71.0; CH_0 δ 160.0, 159.6, 144.9, 139.1, 132.0, 41.1; IR (neat) 2963, 2858, 1725, 1600, 1518, 1427, 1431, 1369, 1288, 1150, 1076, 735, 701 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{38}H_{56}NaO_6Si$ (M+Na): 659.3744, found: 659.3746.



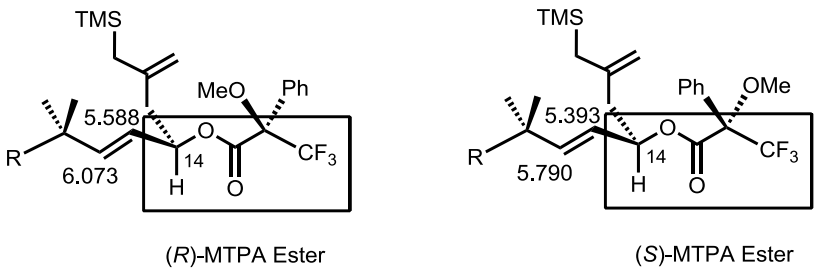
Preparation of (*S*)-(*R*,*E*)-7-((*S*)-2-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-3,4-dihydro-2H-pyran-6-yl)-7-methyl-2-((trimethylsilyl) methyl)octa-1,5-dien-4-yl 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate: To a stirring solution of alcohol **1.68** (9.7 mg, 0.015 mmol, 1.0 equiv) in CH_2Cl_2 (150 µL, 0.1 M) in a 5 mL reaction vial at rt was added DCC (3.8 mg, 0.018 mmol, 1.2 equiv), DMAP (2 mg, 0.015 mmol, 1.0 equiv), and (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid (4.3 mg, 0.018 mmol, 1.2 equiv). The mixture was stirred at rt for 48 h after which the solvent was evaporated under reduced pressure. Purification was accomplished using flash column chromatography (1x10 cm) using 5% EtOAc/hexanes collecting 4 mL fractions. Fractions 12-22 provided the desired product (9.5 mg, 73%) as a colorless liquid. R_f = 0.58 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +1.9 (*c* = 0.35, $CHCl_3$); 500 MHz ^{1}H NMR (C_6D_6) δ 7.78 (d, *J* = 7.8 Hz, 2H), 7.37 (d, *J* = 7.3 Hz, 2H), 7.31 (d, *J* = 8.7 Hz, 2H), 7.21-7.05 (m, 6H), 6.82-6.80 (m, 2H), 6.18 (d, *J* = 15.6 Hz, 1H), 5.89 (ddd, *J* = 8.3, 8.3, 5.8 Hz, 1H), 5.44 (dd, *J* = 15.6, 8.8 Hz, 1H), 4.79 (dd, *J* = 18.5, 6.8 Hz, 1H), 4.75 (d, *J* = 0.9 Hz, 1H), 4.69-4.57 (m, 6H), 4.18-4.14 (m,

1H), 4.08 (q, *J* = 6.3 Hz, 1H), 4.02 (ddd, *J* = 10.2, 4.8, 1.4 Hz, 1H), 3.55 (s, 3H), 3.30 (s, 3H), 2.41 (dd, *J* = 14.6, 8.3 Hz, 1H), 2.23 (dd, *J* = 14.6, 5.3 Hz, 1H), 2.10-1.90 (m, 2H), 1.87-1.80 (m, 1H), 1.61 (ddd, *J* = 13.1, 10.7, 2.4 Hz, 1H), 1.56-1.52 (m, 1H), 1.48 (s, 3H), 1.46-1.36 (m, 1H), 1.27 (s, 3H), 1.24 (s, 3H), 1.23 (s, 3H), -0.02 (s, 9H); 125 MHz ^{13}C NMR (C_6D_6) δ 166.3, 160.1, 159.0, 144.1, 142.9, 139.2, 133.8, 131.9, 129.9, 129.8, 128.9, 128.8, 128.6, 128.3, 128.0, 124.5, 114.4, 111.4, 94.3, 93.8, 78.1, 76.5, 73.8, 73.5, 72.7, 69.7, 56.0, 55.1, 43.7, 41.3, 36.3, 28.8, 27.1, 26.3 (x2), 21.0, 15.4, -1.0; 125 MHz DEPT ^{13}C NMR (C_6D_6) CH_3 δ 56.0, 55.1, 26.3 (x2), 15.4, -1.0; CH_2 δ 111.4, 93.8, 73.5, 69.7, 43.7, 36.3, 28.8, 27.1, 21.0; CH_1 δ 144.1, 129.9, 129.8, 128.9, 128.8, 128.6, 128.0, 94.3, 93.8, 78.1, 76.5, 73.8, 73.5, 72.7, 69.7; CH_0 δ 207.7, 166.3, 160.1, 159.0, 142.9, 139.2, 133.8, 131.9, 41.3; IR (neat) 3077, 3006, 2934, 2812, 1730, 1621, 1430, 1411, 1401, 1305, 1232, 1102, 977, 866, 720, 612 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{48}H_{63}NaF_3O_8Si$ (M+Na): 875.4142, found: 875.4156.

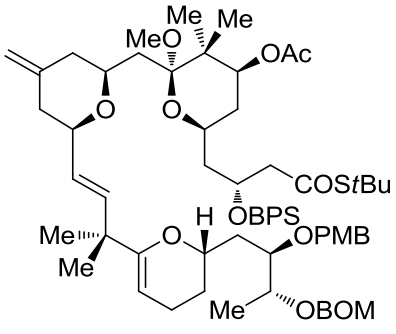


Preparation of (*R*)-(*R*,*E*)-7-((*S*)-2-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-3,4-dihydro-2H-pyran-6-yl)-7-methyl-2-((trimethylsilyl) methyl)octa-1,5-dien-4-yl 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate: This compound was prepared in the similar manner as

that of compound previous compound. R_f = 0.58 (30% EtOAc/hexanes); $[\alpha]_D^{20} = +24$ (*c* = 0.25, $CHCl_3$); 500 MHz ^{1}H NMR (C_6D_6) δ 7.76 (d, *J* = 7.8 Hz, 2H), 7.37 (d, *J* = 7.3 Hz, 2H), 7.30 (d, *J* = 8.7 Hz, 2H), 7.21-7.04 (m, 6H), 6.82-6.79 (m, 2H), 6.22 (d, *J* = 15.6 Hz, 1H), 5.92 (ddd, *J* = 8.3, 8.3, 5.8 Hz, 1H), 5.59 (dd, *J* = 15.6, 8.8 Hz, 1H), 4.81 (dd, *J* = 18.5, 6.8 Hz, 1H), 4.77 (d, *J* = 0.9 Hz, 1H), 4.69-4.56 (m, 6H), 4.17-4.13 (m, 1H), 4.08 (q, *J* = 6.3 Hz, 1H), 4.02 (ddd, *J* = 10.2, 4.8, 1.4 Hz, 1H), 3.54 (s, 3H), 3.30 (s, 3H), 2.44 (dd, *J* = 14.6, 8.3 Hz, 1H), 2.22 (dd, *J* = 14.6, 5.3 Hz, 1H), 2.01-1.79 (m, 2H), 1.61 (ddd, *J* = 13.1, 10.7, 2.4 Hz, 1H), 1.55-1.49 (m, 1H), 1.46 (s, 3H), 1.44-1.36 (m, 2H), 1.27 (s, 6H), 1.23 (d, *J* = 6.3 Hz, 3H), -0.02 (s, 9H); 125 MHz ^{13}C NMR (C_6D_6) δ 166.4, 160.1, 158.9, 144.7, 142.5, 139.1, 138.1, 133.8, 131.9, 129.9, 129.8, 128.9, 128.8, 128.6, 128.3 (x2), 128.0, 124.7, 114.4, 111.5, 94.4, 93.8, 78.1, 76.6, 73.7, 73.5, 72.7, 69.7, 55.8, 55.1, 43.6, 41.4, 36.3, 28.8, 27.2, 26.3 (x2), 21.0, 15.4, -1.0; 125 MHz DEPT ^{13}C NMR (C_6D_6) CH_3 δ 55.8, 55.1, 26.3 (x2), 15.4, -1.0; CH_2 δ 111.5, 93.8, 73.4, 69.7, 43.6, 36.3, 28.8, 27.2, 21.0; CH_1 δ 144.7, 129.9, 129.8, 128.9, 128.8, 128.6, 128.3, 128.0, 124.7,114.4, 94.4, 78.1, 76.6, 73.7, 72.7,; CH_0 δ 166.4, 160.1, 158.9, 142.9, 139.1, 138.1, 133.8, 131.9, 128.3, 41.3; IR (neat) 3072, 3010, 2911, 2850, 1720, 1614, 1411, 1408, 1401, 1355, 1310, 1222, 1102, 1007, 972, 864, 713, 619 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{48}H_{63}NaF_3O_8Si$ (M+Na): 875.4142, found: 875.4156.



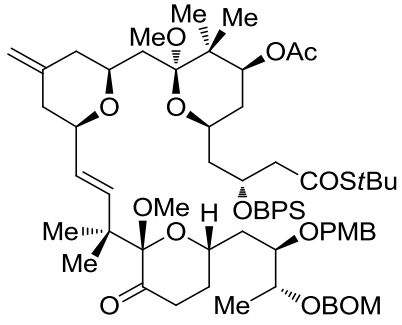
Moscher Ester Analysis of Alcohol 1.68 Shows Desired Steteochemistry at C_{14} Alcohol



Preparation of (2*S*,4*S*,6S)-2-(((2*S*,6*R*)-6-((*E*)-3-((*S*)-2-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-(4-methoxybenzyloxy)butyl)-3,4-dihydro-2H-pyran-6-yl)-3-methylbut-1-enyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-6-((*R*)-2-(*tert*-butyldiphenylsilyloxy)-4-(*tert*-butylthio)-4-oxobutyl)-2-methoxy-3,3-dimethyltetrahydro-2H-pyran-4-yl acetate (1.115): To a stirring solution of aldehyde **1.69** (101 mg, 0.154 mmol, 1.0 equiv) and hydroxyallylsilane **1.68** (108 mg, 0.169 mmol, 1.1 equiv) in Et_2O (2.2 mL) in a flame dried 25 mL rb flask at -78 ºC was added a solution of TMSOTf in Et_2O (200 µL, 0.926 M, 0.184 mmol, 1.2 equiv). After 1.5 h at -78 ºC, the reaction was quenched by addition of diisopropylethylamine (0.2 mL), followed by addition of saturated aqueous $NaHCO_3$ solution (10 mL). The mixture was warmed to rt, the phases were separated, and the aqueous phase was extracted with Et_2O (2 x 15 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 1 x 17 cm silica gel column, eluting with hexanes/EtOAc (9:1), collecting 4 mL

fractions. The product containing fractions (20-55) were combined and concentrated under reduced pressure to provide the pyran **1.115** (107 mg, 58%) as a white foam. The column also furnished a mixture of aldehyde **1.69** and TMS protected silane which were separately purified using Hexanes/ EtOAc (95:5) to give 35 mg (35%) of aldehyde **1.69** and 32 mg (27%) of TMS protected silane. R_f = 0.56 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +18.8 (*c* = 0.65, EtOAc); 500 MHz ^{1}H NMR (C_6D_6) δ 7.83-7.80 (m, 4H), 7.38-7.09 (m, 13H), 6.82-6.79 (m, 2H), 6.08 (dd, *J* = 15.6, 0.9 Hz, 1H), 5.71 (dd, *J* = 16.1, 5.3 Hz, 1H), 5.35 (dd, *J* = 11.7 Hz, 4.8, 1H), 4.88 (s, 1H), 4.82 (d, *J* = 6.8 Hz, 1H), 4.77 (d, *J* = 4.3 Hz, 1H), 4.71-4.66 (m, 3H), 4.64-4.63 (m, 4H), 4.57-4.54 (m, 1H), 4.20-4.16 (m, 1H), 4.08-4.06 (m, 2H), 3.88-3.85 (m, 1H), 3.65-3.63 (m, 1H), 3.30 (s, 3H), 2.93 (s, 3H), 2.91-2.89 (m, 2H), 2.33-2.24 (m, 2H), 2.19-2.15 (m, 1H), 2.11 (t, *J* = 12.4 Hz, 1H), 2.01-1.94 (m, 2H), 1.91-1.76 (m, 4H), 1.72 (d, *J* = 3.9 Hz, 1H), 1.69-1.66 (m, 1H), 1.64 (s, 3H), 1.60-1.52 (m, 4H), 1.43 (s, 9H), 1.34 (s, 3H), 1.33 (s, 3H), 1.23 (d, *J* = 6.3Hz, 3H), 1.19 (s, 9H), 1.07(s, 3H), 1.03 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 198.1, 169.8, 160.0, 159.6, 145.5, 139.2, 138.9, 136.7, 136.6, 135.0, 134.2, 132.0, 130.5, 130.4, 129.9, 128.9, 128.6, 128.4, 128.4, 128.3, 128.0, 114.4, 109.1, 104.7, 94.0, 93.8, 79.1, 78.2, 75.3, 73.9, 73.8, 73.7, 72.5, 70.3, 69.7, 66.9, 55.1, 54.0, 48.5, 48.3, 44.4, 42.9, 42.6, 41.4, 41.2, 39.9, 36.5, 35.5, 30.2, 28.9, 27.6, 26.6, 26.4, 21.2, 21.1, 21.0, 20.0, 17.2, 15.6; 125 MHz DEPT ^{13}C NMR (C_6D_6) CH_3 δ 55.1, 48.5, 30.2, 27.6, 26.6, 26.4, 21.1 (x2), 17.2, 15.6 ; CH_2 δ 109.1, 93.8, 73.6, 69.7, 54.0, 44.4, 42.9, 41.7, 39.9, 36.5, 33.5, 28.9, 21.1; CH δ 138.9, 136.7, 136.6, 130.5 (x2), 129.9, 128.9, 128.4, 128.4, 128.3, 128.0, 114.4, 94.0, 79.1, 78.2, 75.2, 73.8, 72.4, 70.3, 66.9; CH_0 δ 198.0, 169.8, 160.0, 159.6, 145.5, 139.2, 135.0, 134.2, 132.0, 130.4, 104.7, 73.9, 48.3, 42.6; IR (neat) 2959, 2361, 1739, 1681, 1513, 1458,

1365, 1247, 1039, 822, 740, 703 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{71}H_{98}NaO_{12}SSi$ (M+Na): 1225.6446, found: 1225.6462.

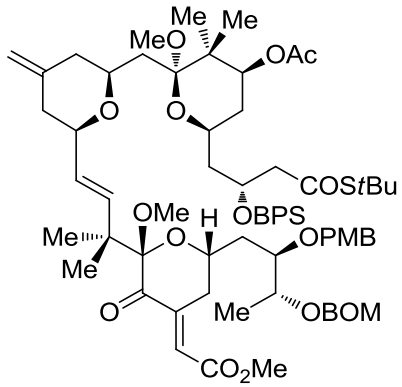


Preparation of (2*S*,4*S*,6*S*)-2-(((2*S*,6*R*)-6-((*E*)-3-((2*S*,6*S*)-6-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-(4-methoxybenzyloxy)butyl)-2-methoxy-3-oxotetrahydro-2H-pyran-2-yl)-3-methylbut-1-enyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-6-((*R*)-2-(*tert*-butyldiphenylsilyloxy)-4-(*tert*-butylthio)-4-oxobutyl)-2-methoxy-3,3-dimethyltetrahydro-2H-pyran-4-yl acetate (1.117): To a stirring solution of dihydropyran **1.115** (130 mg, 0.108 mmol, 1.0 equiv) in CH_2Cl_2 (1.1 mL) at 0 °C, was added MeOH (0.54 mL). Powdered $NaHCO_3$ (13.6 mg, 0.162 mmol, 1.5 equiv) was added in one portion and the solution was stirred at 0 °C for 10 min. Magnesium monoperoxyphthalate (80 %, 80 mg, 0.129 mmol, 1.2 equiv) was added slowly and the mixture was stirred for 30 min at 0°C. The reaction mixture was then quenched by the addition of saturated aqueous $NaHCO_3$ solution (10 mL), then diluted with EtOAc (10 mL) and the layers were separated. The aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layer was washed with brine (30 mL), dried over Na_2SO_4, filtered, concentrated and taken to the next step without further purification.

To a solution of the previously described crude intermediate alcohol in CH_2Cl_2 (2.2 mL), at rt, were added 4 Å molecular sieves (300 mg), TPAP (3.8 mg, 0.01 mmol, 0.1 equiv), and 4-methylmorpholine-N-oxide (38 mg, 0.324 mmol, 3.0 equiv). The

mixture was stirred at rt for 30 min and then diluted with EtOAc (20 mL). The mixture was then filtered through a small plug of Florisil® and washed with copious amounts of EtOAc. The solvent was removed under reduced pressure and purification was accomplished with flash column chromatography, using a 25 x 120 mm silica gel column, eluting with 15% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions from 24 to 75 were combined and concentrated under reduced pressure to provide pure methoxy ketone **1.117** (78 mg, 58% over 2 steps) as a white foam.: R_f = 0.62 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +22 (*c* = 1.0, EtOAc); 500 MHz ^{1}H NMR (C_6D_6) δ 7.81-7.78 (m, 4H), 7.38-7.09 (m, 13H), 6.79-6.77 (m, 2H), 6.30 (d, *J* = 16.1 Hz, 1H), 5.57 (dd, *J* = 4.8, 16.1 Hz, 1H), 5.32 (dd, *J* = 4.3, 11.2 Hz, 1H), 4.85 (s, 1H), 4.78-4.73 (m, 3H), 4.66-4.54 (m, 7H), 4.44-4.41 (m, 1H), 4.15-4.08 (m, 2H), 4.05-4.02 (m, 1H), 3.76-3.73 (m, 1H), 3.59-3.58 (m, 1H), 3.30 (s, 3H), 3.25 (s, 3H), 2.93 (s, 3H), 2.90-2.89 (m, 2H), 2.72-2.08 (m, 5H), 1.99 (ddd, *J* = 17.1, 12.4, 12.4, Hz, 2H), 1.86-1.73 (m, 4H), 1.65 (s, 3H), 1.62-1.52 (m, 3H), 1.42 (s, 9H), 1.37 (s, 3H), 1.35 (s, 3H), 1.21 (d, *J* = 6.3 Hz, 3H), 1.17 (s, 9H), 1.04 (s, 3H), 1.02 (s, 3H); 125 MHz ^{13}C NMR (C_6D_6) δ 205.6, 198.1, 169.8, 160.0, 145.3, 139.0, 137.0, 136.7, 136.5, 135.0, 134.2, 131.7, 130.5, 130.4, 129.8, 129.7, 128.9, 128.4 (x2), 128.3, 128.1,128.1, 114.4, 109.1, 104.7, 104.6, 93.8, 78.8, 77.8, 75.1, 73.8, 72.9, 72.5, 70.4, 69.8, 66.9, 55.1, 54.0, 52.8, 48.5, 48.3, 44.8, 44.3, 42.9, 42.6, 41.6, 39.8, 38.1, 36.7, 33.5, 31.1, 30.2, 27.6, 23.6, 23.0, 21.2, 21.1, 20.0, 17.3, 15.0; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 55.1, 52.8, 48.5, 30.2, 27.6, 23.6, 23.0, 21.2, 21.1, 17.3, 15.0 ; CH_2 δ 109.2, 93.8, 72.4, 69.8, 54.0, 44.3, 42.9, 41.5, 39.9, 38.1, 36.7, 33.5, 31.1; CH δ 137.0, 136.7, 136.6, 130.5, 130.4, 129.8, 129.7, 129.0, 128.6, 128.4 (x2), 128.3, 128.1, 114.4, 78.9, 77.8, 75.1, 73.8, 72.9, 70.4,

66.9; CH_0 δ 205.6, 198.1, 169.8, 160.0, 145.3, 139.0, 135.0, 134.2, 131.7, 104.7, 104.6, 48.3, 44.8, 42.6, 20.0; IR (neat) 3453, 2936, 1734, 1618, 1613, 1588, 1513, 1458, 1428, 1366, 1301, 1246, 1110, 1042, 895, 822, 742, 703 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{72}H_{100}NaO_{14}SSi$ (M+Na): 1271.6501, found: 1271.6491.



Preparation of Methyl (*E*)-methyl 2-((2*S*,6*S*)-2-((*E*)-4-((2*R*,6*S*)-6-(((2*S*,4*S*,6*S*)-4-acetoxy-6-((*R*)-2-(*tert*-butyldiphenylsilyloxy)-4-(*tert*-butylthio)-4-oxobutyl)-2-methoxy-3,3-dimethyltetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)-2-methylbut-3-en-2-yl)-6-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-(4-methoxy benzyloxy)butyl)-2-methoxy-3-oxo-2H-pyran-4(3H,5H,6H)-ylidene)acetate (1.120): To a stirring solution of $(i\text{Pr})_2\text{NH}$ (0.27 mL, 1.93 mmol) in 6 mL of THF in a 25 mL rb flask at -78 ºC was added *n*-BuLi (2.61 M in hexanes, 0.67 mL, 1.75 mmol) via syringe. The solution stirred at -78 ºC for 30 min and was then allowed to warm to 0 ºC for 20 min. This 0.25 M LDA solution was used immediately in the following aldol reaction.

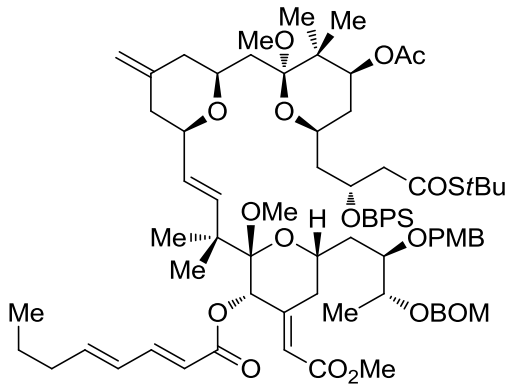
To a stirring solution of ketone **1.117** (102 mg, 0.0816 mmol, 1.0 equiv) in THF (2.7 mL, 0.03 M) in a 10 mL rb flask at -78 ºC was added a 0.25 M solution of LDA in THF (0.35 mL, 0.0897 mmol, 1.1 equiv) slowly via syringe down the side of the flask. The resulting light-yellow reaction mixture was allowed to stir at -78 ºC for 12 min and a

freshly prepared solution of methyl glyoxylate (ca 3.0 M in THF, 0.54 mL, 1.632 mmol, 20.0 equiv) was added slowly via syringe down the side of the flask upon which the yellow color of the solution disappeared. The reaction mixture stirred at -78 ºC for 40 min and was quenched by addition of 2 mL of saturated aqueous NH_4Cl solution. The mixture was allowed to warm to rt and was then partitioned between 10 mL of EtOAc and 10 mL of brine. The phases were separated and the aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 2 x 8 cm silica gel column, eluting with 20% EtOAc/hexanes (100 mL) then 40% EtOAc/hexanes (100 mL), collecting 4 mL fractions. Fractions 6-20 gave unreacted starting material which were combined and concentrated to provide 48 mg of the starting ketone **1.117** (47%). The product containing fractions (22-37) were combined and concentrated under reduced pressure to provide the intermediate aldol adduct as a mixture of diastereomers (53.3 mg, 49%). This material was taken into the following elimination reaction.

To a stirring solution of the aforementioned aldol adduct (31.2 mg, 0.0233 mmol, 1.0 equiv) in CH_2Cl_2 (2.3 mL, 0.01 M) in a 5 mL reaction vial at rt was added diisopropylethylamine (23 µL, 0.1631 mmol, 7.0 equiv), DMAP (2.8 mg, 0.0233 mmol, 1.0 equiv), and carbonyldiimidazole (19 mg, 0.1165 mmol, 5.0 equiv). The reaction mixture was allowed to stir at rt for 24 h and was then quenched by addition of saturated aqueous $NaHCO_3$ solution (5 mL). The mixture was partitioned between EtOAc (10 mL) and brine (10 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over Na_2SO_4, filtered, and

concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 1 x 10 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 4 ml fractions. The product containing fractions (27-54) were combined and concentrated under reduced pressure to provide pure enoate **1.120** (25.3 mg, 82% over 2 steps) as a clear light-yellow oil: R_f = 0.48 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = -8.6 (*c* = 0.53, EtOAc); 500 MHz ^{1}H NMR (C_6D_6) δ 7.84-7.81 (m, 4H), 7.40-7.39 (m, 2H), 7.26-7.11 (m, 11H), 6.79-6.78 (m, 1H), 6.76-6.74 (m, 2H), 6.10 (d, *J* = 16.1 Hz, 1H), 5.50 (dd, *J* = 16.5, 5.3 Hz, 1H), 5.36 (dd, *J* = 11.7, 4.8 Hz, 1H), 4.92-4.80 (m, 3H), 4.74 (dd, *J* = 10.7, 6.8 Hz, 2H), 4.67-4.57 (m, 5H), 4.52 (d, *J* =11.2 Hz, 1H), 4.33 (d, *J* = 11.2 Hz, 1H), 4.18-4.02 (m, 4H), 3.76-3.75 (m, 1H), 3.64-3.61 (m, 1H), 3.36 (s, 3H), 3.28 (s, 3H), 3.24 (s, 3H), 2.95 (s, 3H), 2.93-2.92 (m, 2H), 2.31-2.29 (m, 1H), 2.16-1.79 (m, 5H), 1.89-1.78 (m, 3H), 1.66 (s, 3H), 1.61-1.57 (m, 2H), 1.45 (s, 9H), 1.33 (s, 3H), 1.28 (s, 3H), 1.19 (s, 12H), 1.10 (s, 3H), 1.07 (s, 3H); 125 MHz ^{13}C NMR (C_6D_6) δ 198.1, 197.0, 169.9, 166.4, 160.0, 149.2, 145.4, 139.1, 136.7, 136.6, 135.7, 135.1, 134.2, 131.5, 130.8, 130.5, 130.4, 129.6, 129.0, 128.6, 128.4 (x2), 122.9, 114.4, 109.2, 105.6, 104.6, 94.0, 78.8, 77.3, 75.1, 73.9, 72.6, 71.9, 70.7, 70.5, 69.9, 67.0, 55.1, 54.0, 52.7, 51.6, 48.5, 48.4, 45.4, 44.4, 42.9, 42.7, 40.9, 39.9, 37.1, 36.5, 33.6, 30.2, 27.9, 23.1, 22.2, 21.3, 21.1, 20.0, 18.9, 17.4, 14.8; 125 MHz DEPT ^{13}C NMR (C_6D_6) CH_3 δ 55.1, 52.7, 51.6, 48.5, 30.3, 27.6, 23.0, 22.2, 21.2, 21.1, 17.4, 14.8; CH_2 δ 109.2, 94.0, 71.9, 69.9, 54.0, 44.4, 42.9, 40.9, 39.9, 37.1, 36.5, 33.5; CH δ 136.7, 136.6, 135.7, 130.8, 130.5, 130.4, 129.6, 129.0, 128.6, 128.4, 128.3, 128.1, 122.9, 114.4, 78.8, 77.3, 75.1, 73.9, 72.6, 70.7, 70.4, 67.0; CH_0 δ 198.1, 197.0, 169.9, 166.4, 160.0, 149.2, 145.4, 139.1, 135.1, 134.2, 131.5, 105.6, 104.6, 45.4, 42.7, 20.0, 18.9; IR (neat) 3609, 3583, 3531, 3070, 2956, 2936, 2861, 2362,

1727, 1680, 1614, 1514, 1460, 1384, 1365, 1301, 1247, 1208, 1175, 1108, 1079, 1043, 821, 737, 701, 633 cm^{-1}; HRMS (ESI/ APCI) calcd for $C_{75}H_{102}NaO_{16}SSi$ (M+Na): 1341.6556, found: 1341.6565.

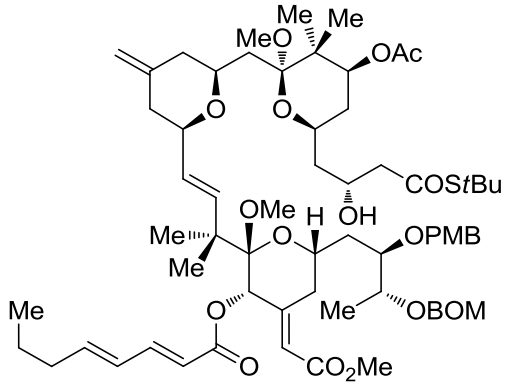


Preparation of (2*E*,4*E*)-((2*S*,3*S*,6*S*,*E*)-2-((*E*)-4-((2*R*,6*S*)-6-(((2*S*,4*S*,6*S*)-4-acetoxy-6-((*R*)-2-(*tert*-butyldiphenylsilyloxy)-4-(*tert*-butylthio)-4-oxobutyl)-2-methoxy-3,3-dimethyltetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)-2-methylbut-3-en-2-yl)-6-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-(4-methoxy benzyloxy)butyl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2H-pyran-3-yl) octa-2,4-dienoate (1.122): To a stirring solution of ketone **1.120** (10.7 mg, 0.0081 mmol, 1.0 equiv) in MeOH (810 µL, 0.01 M) in a 5 mL reaction vial at rt was added $CeCl_3 \cdot 7H_2O$ (60 mg, 0.162 mmol, 20.0 equiv). The mixture was stirred until all the $CeCl_3 \cdot 7H_2O$ was completely dissolved. The mixture was then cooled to -42 ºC and stirred for 10 min and $NaBH_4$ (3.0 mg, 0.081 mmol, 10.0 equiv) was then added. Stirring continued for 2 h at -42 ºC after which another 10 equiv of $NaBH_4$ was added. The mixture was warmed slowly to 0 ºC over 2 h, and then diluted with 40% EtOAc/hexanes. Saturated aqueous NH_4Cl solution (2 mL) was then added. The layers were separated and the aqueous layer was extracted with 40% EtOAc/hexanes (3 x 5mL). The organic phase was washed with brine (5 mL), then dried

over Na_2SO_4, filtered and concentrated under reduced pressure to provide crude intermediate alcohol which was carried directly to the next step without purification.

To a stirring solution of the aforementioned intermediate alcohol in CH_2Cl_2 (810 µL, 0.001 M) in a 5 mL reaction vial at rt was added pyridine (7 µL, 0.081 mmol, 10.0 equiv), DMAP (2.0 mg, 0.016 mmol, 2.0 equiv), and octadienoic anhydride (11.0 mg, 0.040 mmol, 5.0 equiv). The reaction mixture stirred at rt for 12 h and was then quenched by the addition of saturated aqueous $NaHCO_3$ solution (2.0 mL). The mixture was stirred vigorously for 30 min and was then partitioned between CH_2Cl_2 (5 mL) and saturated aqueous $NaHCO_3$ solution (5 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography using 8% EtOAc/hexanes followed by a preparative TLC with 30% EtOAc/hexanes to provide the ester **1.122** (10.2 mg, 87%, 2 steps) as a pale yellow liquid. NMR of the product showed essentially a single diastereomer. R_f = 0.43 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +4.4 (*c* = 0.35, EtOAc); 500 MHz ^{1}H NMR (C_6D_6) δ 7.84-7.81(m, 4H), 7.45 (dd, *J* = 15.1, 10.7 Hz, 1H), 7.40-7.39 (m, 2H), 7.26-7.11 (m, 11H), 6.78-6.76 (m, 2H), 6.39-6.36 (m, 1H), 6.31 (s, 1H), 6.02 (s, 1H), 5.94-5.87 (m, 1H), 5.8 (d, *J* = 15 Hz, 1H), 5.68-5.62 (m, 1H), 5.59 (dd, *J* = 15.6, 4.3 Hz, 1H), 5.37 (dd, *J* = 11.7, 4.8 Hz, 1H), 4.90 (s, 1H), 4.81-4.76 (m, 3H), 4.67 (d, *J* = 12.2 Hz, 1H), 4.63 (d, *J* = 12.2 Hz, 1H), 4.59 (d, *J* = 11.2 Hz, 1H), 4.48 (d, *J* = 11.2 Hz, 1H), 4.34-4.30 (m, 1H), 4.14-4.11 (m, 1H), 4.08-4.05 (m, 1H), 3.83 (s, 2H), 3.66-3.64 (m, 1H), 3.31(s, 6H), 3.30 (s, 3H), 2.97 (s, 3H), 2.92-2.90 (m, 2H), 2.65 (t, *J* = 13.1 Hz, 1H), 2.34-2.25 (m, 2H), 2.20 (dd, *J* = 16.1, 5.3 Hz, 1H), 2.15-2.08 (m, 2H), 1.94-1.70 (m,

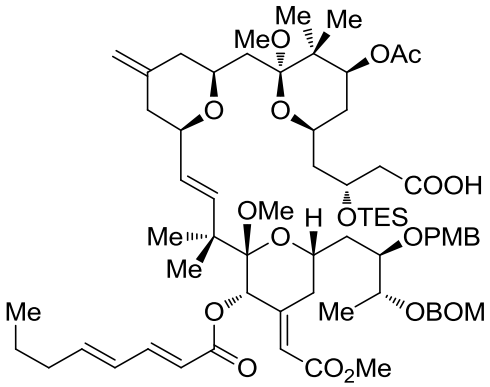
8H), 1.66 (s, 3H), 1.63-1.60 (m, 2H), 1.46 (s, 9H), 1.42 (s, 3H), 1.38 (s, 3H), 1.19 (s, 12H), 1.12 (s, 3H), 1.09 (s, 3H), 0.74 (t, J = 7.3 Hz, 3H); 125 MHz ^{13}C NMR (C_6D_6) δ 198.0, 169.8, 166.7, 165.7, 160.0, 153.9, 146.8, 145.7, 145.3, 139.2, 137.9, 136.7, 136.6, 135.0, 134.2, 131.7, 130.5, 130.4, 129.8, 129.1, 128.9, 128.6, 128.4(x2), 128.3, 128.0, 119.6, 117.8, 117.7, 114.4, 109.1, 104.7, 93.8, 79.0, 77.6, 75.3, 73.9, 72.9, 72.5, 72.3, 70.3, 69.8, 69.3, 66.9, 55.1, 54.0, 51.7, 51.0, 48.6, 48.3, 46.8, 44.5, 43.1, 42.7, 41.2, 39.9, 37.1, 35.5, 34.1, 33.6, 30.2, 27.6, 25.2, 24.6, 22.4, 21.3, 21.1, 20.0, 17.3, 15.0, 14.1; 125 MHz DEPT ^{13}C NMR (C_6D_6) CH_3 δ 55.1, 51.7, 50.9, 48.6, 30.2, 27.6, 25.2, 24.6, 21.3, 21.1, 17.3, 15.0, 14.1; CH_2 δ 109.1, 93.8, 72.3, 69.8, 54.0, 44.5, 43.1, 41.2, 39.9, 37.1, 35.5, 33.6, 22.4; CH δ 146.8, 145.3, 137.9, 136.7, 136.6, 130.5, 130.4, 129.8, 130.5, 130.4, 129.8, 128.9, 128.6, 128.4, 128.2, 128.0, 119.6, 114.4, 78.9, 77.5, 75.3, 73.9, 72.9, 72.4, 70.3, 69.3, 66.9; CH_0 δ 198.0, 169.8, 166.7, 165.7, 160.0, 145.7, 135.0, 134.2, 131.7, 128.4, 117.8, 104.7, 48.3, 46.8, 42.7, 34.1, 20.0; IR (neat) 3069, 2957, 2933, 2361, 1720, 1681, 1643, 1614, 1513, 1459, 1431, 1383, 1364, 1302, 1246, 1131, 1107, 1041, 1003, 891, 859, 821, 737, 702 cm^{-1}; HRMS (ESI/ APCI) calcd for $C_{83}H_{114}NaO_{17}SSi$ (M+Na): 1465.7444, found: 1465.7462.



Preparation of (2*E*,4*E*)-((2*S*,3*S*,6*S*,*E*)-2-((*E*)-4-((2*R*,6*S*)-6-(((2*S*,4*S*,6*R*)-4-acetoxy-6-((*R*)-4-(tert-butylthio)-2-hydroxy-4-oxobutyl)-2-methoxy-3,3-dimethyltetrahydro-2H-pyran-2-yl)methyl)-4-

methylenetetrahydro-2H-pyran-2-yl)-2-methylbut-3-en-2-yl)-6-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-(4-methoxybenzyloxy)butyl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2H-pyran-3-yl) octa-2,4-dienoate (1.124): To a stirring solution of the BPS ether **1.122** (30.2 mg, 0.02 mmol, 1.0 equiv) in a 5:4:1 THF/MeOH/ pyridine (1.0 mL, 0.02M) at 0 ºC in a 15 mL plastic centrifuge tube was added HF·Py (20 %, 0.46 mL). The reaction mixture was stirred at 0 ºC for 30 min and warmed to rt. Stirring continued for 72 h and the reaction mixture was quenched by pipetting into a mixture of sat. aqueous $NaHCO_3$ solution and EtOAc (10 mL each). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 1 x 10 cm silica gel column, eluting with 15% EtOAc/hexanes, collecting 4 ml fractions. The product containing fractions (18-60) were combined and concentrated under reduced pressure to provide alcohol **1.124** (24.3 mg, 96%) as a clear colorless oil: R_f = 0.25 (30% EtOAc/hexanes); $[\alpha]_D^{20} = +10.3$ (*c* = 0.32, EtOAc); 500 MHz ^{1}H NMR (C_6D_6) δ 7.45 (dd, *J* = 15.1, 10.7 Hz, 1H), 7.41-7.39 (m, 3H), 7.26-7.09 (m, 4H), 6.78-6.76 (m, 2H), 6.35 (d, *J* = 16.1 Hz, 1H), 6.31 (s, 1H), 6.01(s, 1H), 5.93 (dd, *J* = 14.6, 10.7 Hz, 1H), 5.86-5.83 (m, 1H), 5.70-5.56 (m, 3H), 4.89 (s, 1H), 4.81-4.77 (m, 3H), 4.68 (d, *J* = 12.2, Hz, 1H), 4.63 (d, *J* = 12.2, Hz, 1H), 4.59 (d, *J* = 11.2 Hz, 1H), 4.48 (d, *J* = 11.2 Hz, 1H), 4.34-4.30 (m, 1H), 4.14-4.11 (m, 1H), 4.08-4.06 (m 1H), 3.90-3.83 (m, 3H), 3.73-3.71 (m, 1H), 3.31 (s, 3H), 3.31 (s, 3H), 3.29 (s, 3H), 3.13 (s, 3H), 2.64 (t, *J* = 14.1 Hz, 1H), 2.53 (dd, *J* = 15.1, 8.3 Hz, 1H), 2.45 (dd, *J* = 15.6, 3.9 Hz, 1H), 2.37 (d, *J* = 13.1 Hz, 1H), 2.31-2.07 (m, 5H), 1.94 (t, *J* = 12.2 Hz, 2H), 1.81-1.71 (m, 5H), 1.70 (s, 3H), 1.52 (d, *J* = 12.2 Hz,

1H), 1.47 (d, *J* = 12.2 Hz, 1H), 1.42 (s, 3H), 1.38 (s, 12H), 1.12-1.18 (m, 6H), 1.12 (s, 3H), 0.74 (t, *J* = 7.3 Hz, 3H); 125 MHz ^{13}C NMR (C_6D_6) δ 199.8, 170.0, 166.7, 165.7, 160.0, 153.9, 146.8, 145.8, 145.3, 139.2, 137.8, 131.6, 129.8, 129.2, 128.9, 128.6, 128.3, 128.0, 127.9, 119.6, 117.8, 114.4, 109.0, 104.6, 93.9, 79.0, 77.5, 75.6, 74.3, 72.9, 72.4, 72.3, 69.8, 69.2, 65.7, 65.5, 55.1, 52.5, 51.7, 51.0, 48.7, 48.5, 46.8, 42.9(x2), 42.5, 41.1, 39.9, 37.0, 35.4, 34.0, 33.4, 30.1, 24.9, 24.8, 22.3, 21.2, 21.1, 17.6, 15.0, 14.0; 125 MHz DEPT ^{13}C NMR (C_6D_6) CH_3 δ 55.1, 51.7, 50.9, 48.7, 30.1, 24.9, 24.8, 21.2, 21.1, 17.6, 15.0, 14.0; CH_2 δ 109.0, 93.9, 72.3, 69.8, 52.5, 42.9, 42.5, 41.1, 39.9, 37.0, 35.4, 34.1, 33.3, 22.3; CH δ 146.8, 145.3, 137.8, 129.8, 129.2, 128.9, 128.3, 128.0, 127.9, 119.6, 117.8, 114.4, 79.0, 77.5, 75.6, 74.2, 72.9, 72.4, 69.2, 65.7, 65.5; CH_0 δ 199.8, 170.0, 166.7, 165.7, 160.0, 153.9, 145.3, 139.2, 131.6, 104.6, 48.5, 46.8, 42.9, 37.0; IR (neat) 3421, 2926, 2361, 1719, 1676, 1643, 1614, 1513, 1456, 1365, 1302, 1248, 1133, 1105, 1041 cm^{-1}; LRMS calcd for $C_{67}H_{96}O_{17}S$ (M+Na): 1228.5237, found: 1228.0.

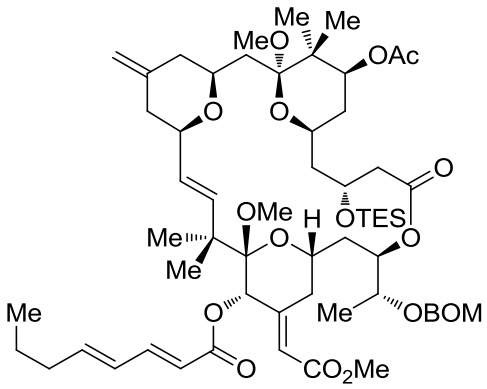


Preparation of (*R*)-4-((2*S*,4*S*,6*S*)-4-acetoxy-6-(((2*S*,6*R*)-6-((*E*)-3-((2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-(4-methoxybenzyloxy)butyl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)-3-((2*E*,4*E*)-octa-2,4-dienoyloxy)tetrahydro-2H-pyran-2-yl)-3-methylbut-1-enyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-6-methoxy-5,5-dimethyltetrahydro-2H-pyran-2-yl)-3-(triethylsilyloxy)butanoic acid (1.126): To a stirring solution of

thiolester **1.124** (6.3 mg, 0.0052 mmol, 1.0 equiv.) in THF (0.4 mL) in a 5 mL vial at 0 ºC was added pH 8 phosphate buffer (0.1 mL). Aqueous lithium hydroxide solution (0.1 M, 104 µL, 0.0104 mmol, 2.0 equiv) was added via syringe followed by 2 drops of 30% H_2O_2 via a 10 µL syringe. The resulting solution stirred at 0 °C for 1 h and another 2 equiv. of LiOH and 2 more drops of H_2O_2 was added. After 1 more h, the reaction mixture was poured into a mixture of pH 6 phosphate buffer solution and EtOAc (10 mL each). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the hydroxy acid as sticky pale yellow oil. The product was taken to the next step without further purification.

To a stirring solution of the after mentioned hydroxy acid in CH_2Cl_2 (0.4 mL) in a 5 mL vial at -15 ºC was added DMAP (3 mg, 0.023 mmol, 4.5 equiv), followed by TESCl (2 µL, 0.013 mmol, 2.5 equiv) via syringe. The solution was stirred at -15 ºC for 1 hr and an additional 2.5 equiv of TESCl was added. The mixture was warmed to 0 ºC over 1 hr after which it was poured into a mixture of aqueous pH 4 (acetic acid/sodium acetate) buffer and EtOAc (10 mL each). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 5 mL). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 1 x 7 cm silica gel column, eluting with 30% EtOAc/hexanes, collecting 2 mL fractions. The product containing fractions (3-9) were combined and concentrated under reduced pressure to provide pure carboxylic acid **1.126** (4.4 mg, 76% over 2 steps) as a colorless oil: R_f = 0.38 (50% EtOAc/hexanes); $[\alpha]_D^{20}$ = +7 (*c* = 0.22, EtOAc); 500 MHz ^{1}H NMR (C_6D_6) δ 7.41-7.39

(m, 3H), 7.26-7.10 (m, 4H), 6.78-6.76 (m, 2H), 6.39 (d, *J* = 15.6 Hz, 1H), 6.32 (s, 1H), 6.02 (s, 1H), 5.95-5.82 (m, 2H), 5.71-5.63 (m, 1H), 5.62-5.58 (m, 2H), 4.86 (s, 1H), 4.83-4.76 (m, 3H), 4.69 (d, *J* = 12.2 Hz, 1H), 4.65(d, *J* = 12.2 Hz, 1H), 4.64-4.59 (m, 2H), 4.50-4.42 (m, 2H), 4.34-4.30 (m, 1H), 4.16-4.12 (m, 1H), 4.09-4.06 (m 1H), 3.90-3.76 (m, 3H), 3.32 (s, 3H), 3.31 (s, 3H), 3.30 (s, 3H), 3.20 (s, 3H), 2.73-2.60 (m, 3H), 2.36-2.21 (m, 3H), 2.14-2.08 (m, 2H), 1.99-1.88 (m, 3H), 1.85-1.1.77 (m, 3H), 1.69 (s, 3H), 1.53 (d, *J* = 12.2, Hz, 1H), 1.48 (d, *J* = 12.2, Hz, 1H), 1.41 (s, 3H), 1.37 (s, 3H), 1.21 (s, 3H), 1.20 (s, 3H), 1.17 (s, 3H), 1.16 (s, 3H), 1.01 (t, *J* = 3.4 Hz, 9H), 0.75 (t, *J* = 7.3 Hz, 3H), 0.66-0.61 (m, 6H); 125 MHz ^{13}C NMR (C_6D_6) δ 170.1, 166.8, 165.8, 160.0, 153.9, 146.8, 145.7, 145.4, 139.0, 138.0, 131.6, 129.8, 129.1, 129.0, 128.4, 128.1, 127.9,128.6, 119.5, 117.8, 114.4, 109.0, 104.9, 93.8, 79.2, 77.5, 75.4, 74.2, 73.1, 72.5, 72.3, 69.8, 69.3, 68.6, 66.5, 55.1, 51.7, 51.0, 48.8, 46.8, 45.1, 44.0, 42.9, 42.8, 41.3, 40.1, 37.1, 35.5, 34.2, 25.1, 24.7, 22.4, 21.4, 21.1, 17.7, 15.0, 14.1, 7.5, 5.9; 125 MHz DEPT ^{13}C NMR (C_6D_6) CH_3 δ 55.1, 51.8, 51.0, 48.8, 25.0, 24.7, 21.4, 21.1, 17.7, 15.0, 14.1, 7.5; CH_2 δ 109.0, 93.8, 72.3, 69.8, 45.1, 44.0, 42.9, 41.3, 40.1, 37.0, 35.5, 34.2, 22.3, 5.9; CH δ 147.0, 145.5, 138.0, 129.8, 129.1, 128.4, 128.1, 127.9, 119.5, 117.8, 114.4, 103.9, 79.2, 77.5, 75.4, 74.2, 73.1, 72.4, 69.3, 68.6, 66.5; CH_0 δ 170.1, 166.8, 165.8, 160.0, 153.9, 145.7, 139.0, 131.6, 129.0, 128.6, 104.9, 46.8, 42.8; IR (neat) 2933, 1733, 1681, 1612, 1513, 1458, 1365, 1246, 1109, 1041, 821, 736, 701 cm^{-1}; LRMS calcd for $C_{69}H_{102}NaO_{18}Si$ (M+Na): 1270.6126, found: 1270.0.

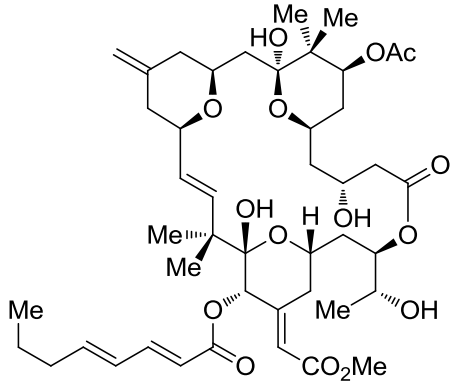


Preparation of (2*E*,4*E*)-(3*S*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*S*,25*S*)-25-acetoxy-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-1,11-dimethoxy-13-(2-methoxy-2-oxoethylidene)-10,10,26,26-tetramethyl-5-methylene-19-oxo-21-((triethylsilyl)oxy)-18,27,28,29-tetraoxatetracyclo [21.3.1.13,7.111,15]nonacos-8-en-12-yl octa-2,4-dienoate (1.128): To a solution of the PMB ether **1.126** (3.1 mg, 0.0024 mmol, 1 equiv) in CH_2Cl_2 (100 µL) in a 5 mL vial at 0 ºC was added pH 8 phosphate buffer (100 µL) and t-butanol (50 µL) via syringe. To the solution was added DDQ (2.8 mg, 0.0124 mmol, 5 equiv) in one portion and the reaction was stirred vigorously for 1 h after which another 5 equiv DDQ was added. After stirring 1 more h at 0 ºC, the reaction mixture was poured into a mixture of CH_2Cl_2 and pH 4 acetate buffer (5 mL each). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was quickly passed through a column of silica gel (1 x 7 cm) eluting with 30% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions 4-11 were combined and concentrated under reduced pressure to give seco acid (2.1 mg) partially mixed with DDQ byproducts which was taken to the next step without further purification.

To a stirring solution of the seco acid in THF (60 µL) in a 5 mL vial at 0 °C was added a 0.1 M solution of triethylamine in THF (112 µL, 0.011 mmol, 6.0 equiv) and a

0.1M solution of 2,4,6-trichlorobenzoyl chloride in THF (112 µL, 0.0055 mmol, 3.0 equiv). After 10 min, the reaction was warmed to rt and stirring continued for an additional 3 h. The reaction mixture was diluted with 3:1 toluene/ THF (1 mL) and taken into a 25 mL gas-tight syringe. This solution was added by syringe pump to a stirring solution of DMAP (4.5 mg, 0.037 mmol, 20.0 equiv) in toluene (1.2 mL) at 40 °C over 12 h. The residual contents of the syringe were rinsed into the flask with toluene (0.5 mL) and stirring continued for an additional 2 h. The reaction mixture was cooled to rt and diluted with 30% EtOAc/hexanes (10 mL) and washed with saturated aqueous $NaHCO_3$ solution (2 x 10 mL) and brine (2 x 5 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 1 x 4 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 2 mL fractions. The product containing fractions (13-25) were combined and concentrated under reduced pressure to provide pure macrolactone **1.128** as a white powder (1.7 mg, 62% over 2 steps): R_f = 0.48 (30% EtOAc/hexanes); $[\alpha]_D^{20} = +9$ (*c* = 0.085, EtOAc); 500 MHz ^{1}H NMR (C_6D_6) δ 7.46 (dd, *J* = 15.1, 10.7 Hz, 1H), 7.33-7.07 (m, 5H), 6.74 (d, *J* = 16.1 Hz, 2H), 6.51 (d, *J* = 1.4 Hz, 1H), 5.92-5.87 (m, 1H), 5.83-5.78 (m, 3H), 5.71 (dd, *J* = 11.7, 4.8 Hz, 1H), 5.63-5.55 (m, 2H), 4.73-4.52 (m, 10H), 4.35-4.31 (m, 2H), 4.05-3.96 (m, 4H), 3.74-3.71 (m, 1H), 3.30 (s, 3H), 3.27 (s, 3H), 3.14 (s, 3H), 2.70 (dd, *J* = 17.5, 3.4 Hz, 1H), 2.47-2.42 (m, 1H), 2.36-2.31 (m, 1H), 2.27-2.18 (m, 2H), 2.12-2.04 (m, 2H), 1.99-1.91 (m, 3H), 1.84-1.80 (m, 2H), 1.73 (s, 3H), 1.54 (s, 3H), 1.28 (s, 3H), 1.21 (s, 3H), 1.13 (d, *J* = 7.3 Hz, 1H), 1.10 (d, *J* = 7.3 Hz, 1H), 1.09 (s, 3H), 1.03 (d, *J* = 6.3 Hz, 3H), 1.00 (t, *J* = 7.8 Hz, 9H), 0.70 (t, *J* = 7.3 Hz, 3H), 0.64-0.59 (m, 6H); 125 MHz ^{13}C NMR (C_6D_6) δ 171.2, 170.1,

167.0, 165.7, 152.2, 147.1, 145.6 (x2), 140.1, 138.9, 128.9 (x2), 128.7, 128.6, 120.5, 119.4, 108.9, 104.1, 103.8, 93.8, 80.6, 74.5, 74.4, 74.2, 74.0, 71.5, 69.9, 67.7, 66.5, 65.3, 53.7, 51.1, 48.6, 45.9, 45.6, 44.0, 42.4 (x2), 42.1, 41.6, 40.7, 36.9, 35.4, 34.7, 31.9, 30.5, 27.6, 22.3, 21.1 (x2), 18.1, 16.2, 14.0, 7.6, 6.3; 125 MHz DEPT ^{13}C NMR (C_6D_6) CH_3 δ 53.7, 51.1 (x2), 48.6 (x2), 21.1 (x2), 18.1, 16.1, 14.0, 7.6; CH_2 δ 108.8, 93.8, 69.9, 45.9, 44.0, 42.1, 40.7, 36.9, 35.4, 34.7, 31.9, 30.5, 22.3, 6.3; CH δ 147.1, 145.5, 140.1, 129.0, 128.9, 128.4, 128.1, 120.5, 119.4, 80.6, 74.5, 74.4, 74.2, 74.0, 71.5, 67.7, 66.5, 65.3; CH_0 δ 171.2, 170.1, 167.0, 165.7, 152.2, 145.6, 128.9, 128.7, 104.1, 45.6, 42.4; IR (neat) 3674, 3526, 1996, 1870, 1846, 1650, 1520, 1159, 819, 789 cm^{-1}; LRMS calcd for $C_{61}H_{92}O_{16}Si$ (M+Na): 1132.4489, found: 1132.1.



Preparation of C_{30}-Decarbomethoxy Bryostatin 1(Merle 28): To a 2 mL reaction vial containing the analogue precursor **1.128** (1.6 mg, 0.00144 mmol, 1 equiv) was added a 0.25 M solution of $LiBF_4$ in 25:1 CH_3CN/ H_2O (260 µL, 0.0648 mmol, 45.0 equiv). The reaction vial was sealed and the mixture was allowed to stir at 80 ºC for 24 h. After cooling to rt, the reaction mixture was diluted with EtOAc (5 mL) and was quenched with a saturated aqueous $NaHCO_3$ solution (5 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated. Purification was accomplished using flash column chromatography with a 0.5 x 6 cm

silica gel column, eluting with 20% EtOAc/hexanes, collecting 6 x 50 mm test tube fractions (1-10) followed by 50% EtOAc/hexanes. The product containing fractions (20-32) were combined and concentrated under reduced pressure to provide Merle 28 (1.0 mg, 83%) as white solid: R_f = 0.25 (50% EtOAc/hexanes; $[\alpha]_D^{20}$ = +4 (*c* = 0.1, EtOAc); 500 MHz ^{1}H NMR ($CDCl_3$) δ 6.18-6.16 (m, 1H), 6.01 (d, *J* = 1.9 Hz, 1H), 5.81 (d, *J* = 11.7 Hz, 1H), 5.78 (d, *J* = 12.2 Hz, 1H), 5.30 (dd, *J* = 15.6, 8.3 Hz, 1H), 5.25 (s, 1H), 5.20 (s, 1H), 5.16 (dd, *J* = 11.7, 4.3 Hz, 1H), 4.75 (d, *J* = 7.8 Hz, 2H), 4.29-4.16 (m, 3H), 4.06-4.01 (m, 1H), 3.85-3.82 (m, 1H), 3.71-3.62 (m 3H), 3.67 (s, 3H), 2.51-2.43 (m, 2H), 2.39 (s, 1H), 2.18-2.13 (m, 2H), 2.10-2.07 (m, 2H), 2.05 (s, 3H), 2.00-1.94 (m, 3H), 1.86-1.81 (m, 1H), 1.79-1.76 (m, 1H), 1.79-1.76 (m, 1H), 1.69-1.62 (m, 2H), 1.57 (s, 3H), 1.51-1.44 (m, 3H), 1.26-1.23 (m, 6H), 1.51 (s, 3H), 1.00 (s, 6H), 0.96 (s, 3H), 0.92 (t, *J* = 7.3 Hz, 3H); 125 MHz ^{13}C NMR (C_6D_6) δ 172.7, 170.3, 167.1, 165.9, 146.9, 145.3, 144.5, 139.8, 130.7, 128.9, 128.7, 128.6, 120.8, 119.7, 109.2, 102.2, 100.2, 80.6, 75.3, 74.4, 73.2, 72.3, 70.6, 69.1, 66.0, 65.7, 50.9, 45.8, 43.2, 42.8, 42.7, 42.0, 40.2, 36.6, 35.4, 33.9, 32.4, 25.7, 22.3, 21.5, 21.0, 20.4, 20.2, 17.2, 14.0; 125 MHz DEPT ^{13}C NMR (C_6D_6) CH_3 δ 50.9, 25.7, 21.5, 21.0, 20.4, 20.2, 17.2, 14.0; CH_2 δ 109.2, 43.2, 42.8, 42.7, 42.0, 40.2, 36.6, 35.4, 33.9, 32.4, 22.3; CH δ 146.9, 145.3, 139.8, 130.7, 129.1, 120.8, 119.7, 80.6, 75.3, 74.4, 73.2, 72.3, 70.6, 69.2, 66.0, 65.7; CH_0 δ 172.7, 170.3, 167.1, 165.9, 144.5, 128.7, 128.6, 102.2, 100.2, 45.8; IR (neat) 3608, 3583, 2932, 2360, 2339, 1736, 1680, 1515, 1459, 1386, 1246, 1109, 820, 663 cm^{-1}; LRMS calcd for $C_{45}H_{66}O_{15}$ (M+Na): 869.4299, found: 869.1.

Biological Experiments and Data for Merle 28

[^{3}H]PDBu binding assay[75]**.** The inhibitory dissociation constant (K*i*) of Merle 28 was determined by the ability of the ligand to displace bound [20-^{3}H]phorbol 12,13-dibutyrate (PDBu) from mouse recombinant isozyme PKC á in the presence of calcium and phosphatidylserine, using a polyethylene glycol precipitation assay previously described by Blumberg and Lewin. Briefly, the assay mixture (250 µL) contained 50 mM Tris-HCl (pH 7.4 at room temperature), 100 µg/mL phosphatidylserine, 0.1 mM Ca^{2+}, 4 mg/mL bovine immunoglobulin G and .003% Tx-100, 2 nM [^{3}H]PDBu and various concentrations of the competing ligand. The assay tubes were incubated at 37°C for 5 min, then chilled for 10 min on ice, after which 200 µL of 35% polyethylene glycol 6000 in 50 mM Tris-HCl (pH 7.4) was added. The tubes were vortexed and chilled an additional 10 min and then centrifuged in a Beckman Allegra 21R centrifuge at 4°C (12,200 rpm, 15 min). A 100 µL aliquot of each supernatant was removed and placed in a scintillation vial for the determination of the free concentration of [^{3}H]PDBu. Each assay pellet, located in the tip of the assay tube, was carefully dried, cut off, and placed in a scintillation vial for the determination of the total bound [^{3}H]PDBu. The radioactivity was determined by scintillation counting, using Cytoscint (ICN, Costa Mesa, CA). Specific binding was calculated as the difference between total and nonspecific PDBu binding. The inhibitory dissociation constant (K_i) was calculated using the method previously described by Blumberg and Lewin. The K*i* for Merle 28 was found to be 0.52 ± 0.06 nM (average of three determinations).

Attachment and proliferation of U937 cells[76] U937 cells (Figure **1.67**), purchased from ATCC (Manassas, VA) and cultured in RPMI-1640 medium supplemented with 10 % FBS (ATCC, Manassas, VA), were plated in 35 mm dishes at a density of 1 X 10^5 living cells/ml and treated with different concentrations of the drugs or DMSO. After 72 h, the number of cells in the supernatant (nonattached cells) and the number of attached cells (after trypsinization) were counted using a particle counter. The number of attached cells is expressed as percent of total cells.

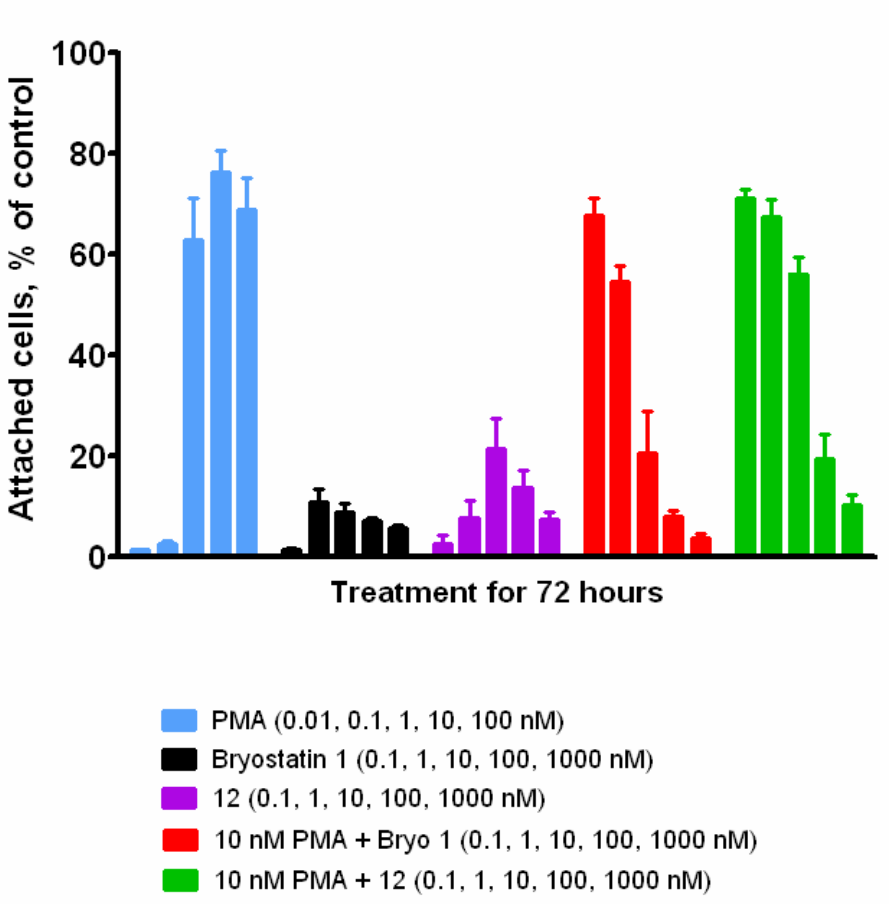


Figure 1.67. Attachment Assay for Merle 28

U937 cells (Figure **1.68**) were treated with PMA (0.01-100 nM), bryostatin1 (0.1-1000 nM), analogue 12 (0.1-1000 nM), 10 nM PMA with different concentrations of bryostatin 1 (0.1-1000 nM) or 10 nM PMA with different concentrations of analogue 12 (0.1-1000 nM). The numbers of attached and nonattached cells were counted and the number of total cells was expressed as % of control. The bars and error bars represent the average and the standard error of the mean of at least three independent experiments.

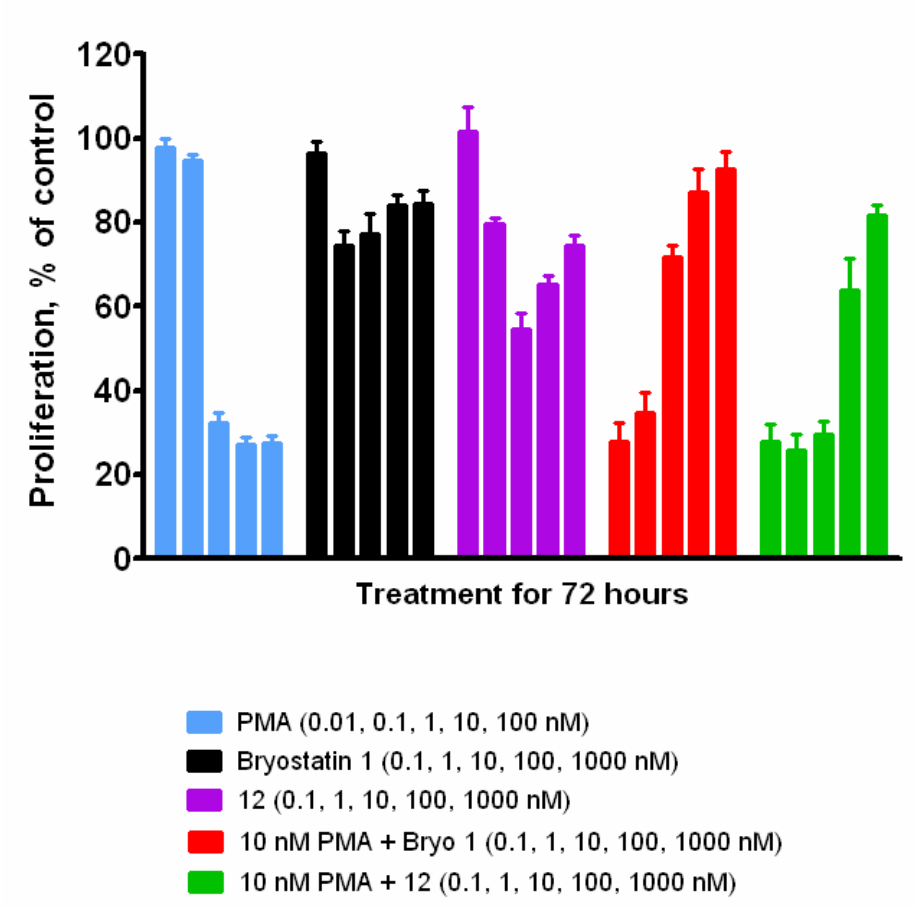
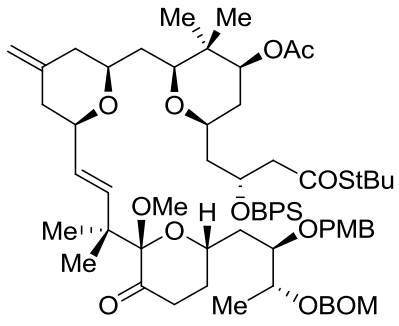


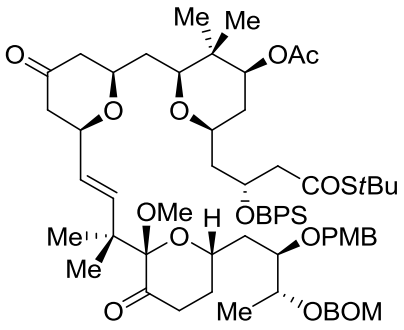
Figure 1.68. Inhibition of Proliferation Assay for Merle 28

Experimental Procedures and Analytical Data for Merle 30



Preparation of (2*S*,4*S*,6*S*)-2-(((2*S*,6*R*)-6-((*E*)-3-((2*S*,6*S*)-6-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-(4-methoxybenzyloxy)butyl)-2-methoxy-3-oxotetrahydro-2H-pyran-2-yl)-3-methylbut-1-enyl)-4-methylene tetrahydro-2H-pyran-2-yl)methyl)-6-((*R*)-2-(*tert*-butyldiphenylsilyloxy)-4-(*tert*-butylthio)-4-oxobutyl)-3,3-dimethyltetrahydro-2H-pyran-4-yl acetate (1.131): To a stirring solution of bismethylketal **1.117** (97 mg, 0.077 mmol, 1.0 equiv) in CH_2Cl_2 (1.6 mL) in a 10 mL rb flask at -78 ºC was added triethylsilane (248 µL, 1.55 mmol, 20 equiv) followed by a solution of TMSOTf in Et_2O (251 µL, 0.92 M, 0.23 mmol, 3 equiv). After 1 h at -78 ºC, the reaction was quenched by addition of saturated aqueous NH_4Cl solution. The mixture was warmed to rt and diluted with CH_2Cl_2 (10 mL). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 x 10 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 1 x 11 cm silica gel column, eluting with hexanes/EtOAc (9:1), collecting 4 mL fractions. The product containing fractions (30-48) were combined and concentrated under reduced pressure to provide the pyran **1.131** as a single diastereomer as a white foam (77 mg, 82%). R_f = 0.40 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +22 (*c* = 1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.67 (m, 4H), 7.45-7.20 (m, 13H), 6.85-6.83 (m, 2H), 6.02 (d, *J* =

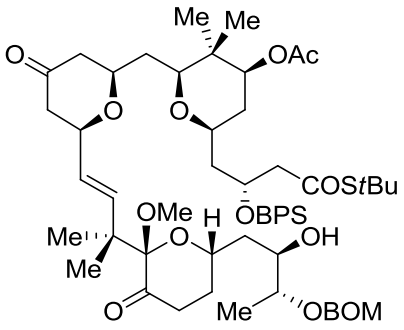
15.6 Hz, 1H), 5.46 (dd, *J* = 15.6, 5.8 Hz, 1H), 4.86 (d, *J* = 6.8 Hz, 1H), 4.84 (d, *J* = 6.8 Hz, 1H), 4.71-4.70 (m, 2H), 4.65 (s, 2H), 4.64-4.61 (m, 1H), 4.47-4.43 (m, 2H), 4.62 (ddd, *J* = 7.0, 5.3, 5.3 Hz, 1H), 4.17-4.12 (m, 1H), 4.11-4.06 (m, 1H), 3.87 (ddd, *J* = 10.2, 4.8, 1.9 Hz, 1H), 3.80-3.76 (m, 1H), 3.79 (s, 3H), 3.45-3.41 (m, 1H), 3.23 (s, 3H), 3.06-3.01 (m, 1H), 2.87-2.85 (m, 1H), 2.66 (dd, *J* = 14.6, 6.8 Hz, 1H), 2.62 (dd, *J* = 14.1, 5.3 Hz, 1H), 2.50-2.38 (m, 2H), 2.20-2.18 (m, 2H), 2.09 (s, 3H), 1.95-1.85 (m, 4H), 1.80-1.63 (m, 5H), 1.43 (s, 3H), 1.33 (dd, *J* = 4.7, 2.3 Hz, 1H), 1.30 (dd, *J* = 4.8, 2.4 Hz, 1H), 1.21 (d, *J* = 6.3 Hz, 3H), 1.13 (s, 3H), 1.09 (s, 3H), 1.02 (s, 9H), 0.79 (s, 3H), 0.72 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 207.2, 197.7, 170.6, 159.3, 163.5, 137.9, 137.0, 136.0, 135.9, 134.4, 133.7, 130.6, 129.8, 129.7, 129.3, 129.1, 128.5, 127.9, 127.8, 127.7(x2), 113.9, 109.5, 104.0, 93.5, 79.6, 79.0, 77.2, 77.0, 75.5, 72.9, 72.6, 72.1, 69.7, 69.5, 69.4, 55.3, 52.6, 52.3, 47.9, 44.2, 43.6, 41.1, 39.5, 37.6, 37.4, 36.3, 35.8, 33.8, 30.2, 29.9, 27.1, 22.9, 22.3, 21.8, 21.2, 19.5, 14.8, 13.5; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 55.3, 52.3, 29.9, 27.1, 22.9, 22.3, 21.8, 21.2, 14.8, 13.5; CH_2 δ 109.5, 93.4, 72.1, 69.5, 52.6, 43.6, 41.1, 39.5, 37.6, 36.3, 35.7, 33.8, 30.2; CH_1 δ 137.0, 135.9 (x2), 129.8, 129.7, 129.3, 129.1, 128.5, 127.8, 127.7, 127.6, 113.9, 79.6, 79.0, 77.2, 77.0, 75.5, 72.9, 72.5, 69.7, 69.4 CH_0 δ 207.2, 197.7, 170.6, 159.3, 143.5, 137.9, 134.4, 133.7, 130.6, 104.0, 47.9, 44.2, 37.4, 19.5; IR (neat) 3070, 2959, 2858, 1741, 1722, 1680, 1473, 1427, 1388, 1241, 1111, 1073, 1029, 979, 822, 739, 704, 682, 611 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{71}H_{98}NaO_{13}SSi$ (M+Na): 1241.6395, found: 1241.6405.



Preparation of (2*S*,4*S*,6*S*)-2-(((2*R*,6*R*)-6-((*E*)-3-((2*S*,6*S*)-6-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-(4-methoxybenzyloxy)butyl)-2-methoxy-3-oxotetrahydro-2H-pyran-2-yl)-3-methylbut-1-enyl)-4-oxotetrahydro-2H-pyran-2-yl)methyl)-6-((R)-2-(*tert*-butyldiphenylsilyloxy)-4-(*tert*-butylthio)-4-oxobutyl)-3,3-dimethyltetrahydro-2H-pyran-4-yl acetate (1.136): In a 100 ml flask, 30 mL of CH_2Cl_2 was cooled to -78 °C, and a stream of O_3 was passed in until the color changed to light blue (5 min). The flask was sealed and kept at -78 °C for immediate use. The O_3 solution prepared above was added 50 μL at a time via a plastic syringe to a stirring solution of olefin **1.117**(35 mg, 0.028 mmol, 1.0 equiv) at -78 °C. The reaction was monitored by TLC and the addition of the O_3 solution was continued every 10 min until the starting material was fully consumed. Dimethyl sulfide (1 mL) was then added and the mixture was allowed to warm to rt.

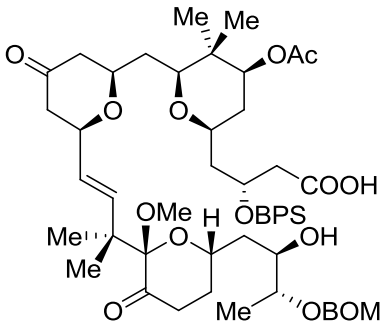
The solution was stirred at rt for 12 h after which the solvent was removed under reduced pressure. Purification was accomplished via flash column chromatography, using a 1 x 7 cm silica gel column, eluting with 20% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (17 to 30) were combined and concentrated under reduced pressure to provide ketone **1.136** (31 mg, 89%) as a white foam. R_f = 0.46 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +4 (*c* = 1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.67 (m, 4H), 7.45-7.27 (m, 11H), 7.22-7.21 (m, 2H), 6.84-6.83

(m, 2H), 6.05 (d, *J* = 16.1 Hz, 1H), 5.46 (dd, *J* = 16.1, 6.3 Hz, 1H), 4.85 (dd, *J* = 11.7, 7.3 Hz, 2H), 4.65 (s, 2H), 4.62 (d, *J* = 11.2 Hz, 1H), 4.45-4.43 (m, 1H), 4.40 (dd, *J* = 11.7, 4.3 Hz, 1H), 4.25 (q, *J* = 4.8 Hz, 1H), 4.18-4.14 (m, 1H), 4.10-4.08 (m, 1H), 3.86 (ddd, *J* = 10.1, 4.3, 1.8 Hz, 1H), 3.78 (s, 3H), 3.75-3.71 (m, 1H), 3.22 (s, 3H), 2.94-2.91 (m, 1H), 2.76-2.60 (m, 3H), 2.50-2.26 (m, 4H), 2.07 (dd, *J* = 14.1, 11.7 Hz, 1H), 2.02 (s, 3H), 1.92-1.84 (m, 4H), 1.70-1.63 (m, 3H), 1.56 (dd, *J* = 12.6, 9.7 Hz, 2H), 1.43 (s, 9H), 1.32-1.31 (m, 2H), 1.21 (d, *J* = 6.3 Hz, 3H), 1.13 (s, 3H), 1.11 (s, 3H), 1.02 (s, 9H), 0.78 (s, 3H), 0.71 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 207.0, 206.1, 197.8, 170.6, 159.3, 138.3, 138.0, 136.1, 136.0, 134.4, 133.7, 130.6, 129.9, 129.8, 129.4, 128.6, 127.9 (x2), 127.8, 127.7, 114.0, 103.9, 93.6, 79.2, 77.4, 77.3, 76.8, 74.1, 73.1, 72.6, 72.2, 69.7, 69.6, 69.1, 55.4, 53.0, 52.5, 48.1, 47.8, 46.6, 44.4, 43.6, 37.7, 37.5, 36.3, 36.0, 33.5, 30.4, 30.0, 29.8, 27.1, 23.0, 22.3, 21.8, 21.2, 19.6, 14.9, 13.5; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 55.4, 52.5, 29.9, 27.1, 23.0, 22.3, 21.8, 21.3, 14.9, 13.5; CH_2 δ 93.6, 72.2, 69.6, 53.0, 47.8, 46.6, 43.6, 37.7, 36.3, 36.0, 33.5, 30.4, 29.8; CH_1 δ 138.3, 136.1, 136.0, 129.9, 129.8, 129.4, 128.6, 127.9 (x2), 127.8, 127.7, 114.0, 79.2, 77.4, 77.3, 76.8, 74.1, 73.1, 72.6, 69.7, 69.1; CH_0 δ 207.0, 206.1, 197.8, 170.6, 159.3, 138.0, 134.4, 133.7, 130.6, 103.9, 48.1, 44.4, 37.5, 19.6; IR (neat) 2959, 2932, 2360, 2339 1723, 1683, 1514, 1456, 1363, 1247, 1111, 1041, 822, 741, 703 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{71}H_{96}NaO_{14}SSi$ (M+Na): 1243.6188, found: 1243.6194.



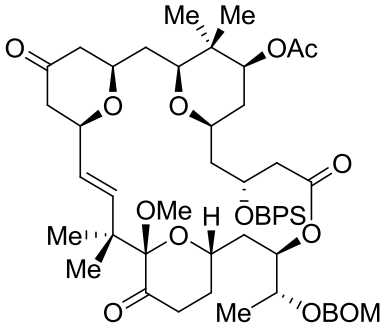
Preparation of (2*S*,4*S*,6*S*)-2-(((2*R*,6*R*)-6-((*E*)-3-((2*S*,6*S*)-6-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-hydroxybutyl)-2-methoxy-3-oxotetrahydro-2H-pyran-2-yl)-3-methylbut-1-enyl)-4-oxotetrahydro-2H-pyran-2-yl)methyl)-6-((*R*)-2-(*tert*-butyldiphenylsilyloxy)-4-(*tert*-butylthio)-4-oxobutyl)-3,3-dimethyltetrahydro-2H-pyran-4-yl acetate (1.140): To a stirring solution of the PMB ether **1.117** (51 mg, 0.041 mmol, 1 equiv) in CH_2Cl_2 (3.2 mL) in a 15 mL flask at 0 ºC was added pH 7 phosphate buffer (800 µL). To the solution was added DDQ (47 mg, 0.20 mmol, 5 equiv) in one portion and the mixture was stirred vigorously for 1 h after which another 5 equiv DDQ was added. After stirring 1 more h at 0 ºC, the reaction was quenched by adding saturated aq. $NaHCO_3$ solution (1 mL) and stirring for 15 min. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated. Purification was accomplished with flash column chromatography, using a 1 x 7 cm silica gel column, eluting with 30% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (25 to 50) were combined and concentrated under reduced pressure to provide alcohol **1.140** (42 mg, 91%) as a white foam. R_f = 0.3 (50% EtOAc/hexanes); $[\alpha]_D^{20}$ = -9.6 (*c* = 0.7, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.66 (m, 4H), 7.45-7.29 (m, 11H), 6.02 (d, *J* = 16.1 Hz, 1H), 5.46 (dd, *J* = 16.1, 6.3 Hz, 1H), 4.89 (d, *J* = 6.8 Hz, 1H), 4.85 (d, *J* = 7.3 Hz, 1H), 4.68 (d, *J* = 11.7 Hz, 1H), 4.64 (d, *J* = 11.7 Hz, 1H), 4.40 (dd, *J*

= 11.7, 4.8 Hz, 1H), 4.28-4.24 (m, 2H), 4.11-4.07 (m, 1H), 3.83 (ddd, *J* = 12.6, 6.3, 3.9 Hz, 1H), 3.77-3.71 (m, 1H), 3.61 (q, *J* = 6.3 Hz, 1H), 3.33 (s, 3H), 2.93 (dddd, *J* = 10.9, 7.5, 4.1, 2.3 Hz, 1H), 2.81-2.80 (m, 1H), 2.75 (dd, *J* = 10.2, 1.4 Hz, 1H), 2.68 (dd, *J* = 14.6, 16.8 Hz, 1H), 2.62 (dd, *J* = 14.1, 4.8 Hz, 1H), 2.57-2.50 (m, 1H), 2.41-2.26 (m, 4H), 2.08 (dd, *J* = 14.1, 11.7 Hz, 1H), 2.02 (s, 3H), 1.92-1.83 (m, 3H), 1.70-1.55 (m, 5H), 1.43 (s, 9H), 1.31 (ddd, *J* = 12.6, 4.3, 1.9 Hz, 2H), 1.25 (d, *J* = 6.3 Hz, 3H), 1.15 (s, 3H), 1.10 (s, 3H), 1.02 (s, 9H), 0.78 (s, 3H), 0.71 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 206.8, 206.2, 197.8, 170.6, 138.4, 137.6, 136.1, 136.0, 134.4, 133.7, 130.0, 129.8, 128.7, 128.1, 128.0, 127.9, 127.8, 127.6, 103.8, 94.0, 79.3, 78.4, 77.4, 76.8, 74.2, 73.1, 71.3, 70.1, 69.6, 69.2, 53.0, 52.0, 48.2, 47.9, 46.7, 44.2, 43.6, 39.5, 37.5, 37.4, 36.0, 33.6, 30.3, 30.0, 27.1, 22.9, 22.4, 22.2, 21.3, 19.6, 17.0, 13.5; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 52.0, 30.0, 27.1, 22.9, 22.4, 22.2, 21.3, 17.0, 13.5; CH_2 δ 94.0, 70.1, 53.0, 47.9, 46.7, 43.6, 39.5, 37.4, 36.0, 33.5, 30.3; CH_1 δ 138.4, 136.1, 136.0, 129.9, 129.8, 128.6, 128.0, 127.9, 127.7, 127.5, 79.2, 78.4, 77.4(x2), 76.8, 74.2, 73.1, 71.3, 69.6, 69.1; CH_0 δ 206.8, 206.2, 179.8, 170.6, 137.6, 134.4, 133.7, 128.1, 103.8, 48.2, 44.2, 37.5, 19.6; IR (neat) 3046, 2960, 2931, 2858, 2360, 2337, 1718, 1678, 1540, 1472, 1456, 1419, 1363, 1244, 1111, 1043, 822, 741, 702, 667 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{62}H_{88}NaO_{13}SSi$ (M+Na): 1123.5613, found: 1123.5614.



Preparation of (*R*)-4-((2*S*,4*S*,6*S*)-4-acetoxy-6-(((2*R*,6*R*)-6-((*E*)-3-((2*S*,6*S*)-6-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-hydroxybutyl)-2-methoxy-3-oxotetrahydro-2H-pyran-2-yl)-3-methylbut-1-enyl)-4-oxotetrahydro-2H-pyran-2-yl)methyl)-5,5-dimethyltetrahydro-2H-pyran-2-yl)-3-(*tert*-butyldiphenylsilyloxy) butanoic acid (1.141): To a stirring solution of thiolester **1.140** (71 mg, 0.064 mmol, 1.0 equiv) in a 4:1 mixture of THF/H_2O (3.2 mL, 0.02 M) at 0 ºC was added *m*CPBA (44 mg, 0.257 mmol, 4.0 equiv). The mixture was stirred at 0 ºC for 1 hr and at rt for 8 h after which TLC showed the completion of reaction. The reaction was diluted with EtOAc (15 ml) and washed with saturated aq. $NaHSO_3$ solution (5 mL) and brine (5 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to provide the crude seco acid. Purification was accomplished using flash column chromatography using 1 x 10 cm silica gel column using 30% EtOAc/hexanes (50 mL) which removed *m*CPBA. Elution of the column with a mixture of 50% hexanes, 30% EtOAc, and 20% MeOH, collecting 4 mL fractions, provided the desired seco acid **1.141** (fractions 21 to 50) (55 mg, 83%) as a white foam. R_f = 0.7 (2:2:1 EtOAc/hexanes/MeOH); $[\alpha]_D^{20}$ = -2.1 (*c* = 1.2, EtOAc); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.68-7.65 (m, 4H), 7.46-7.29 (m, 9H), 6.09 (d, *J* = 16.1 Hz, 1H), 5.45 (dd, *J* = 15.6, 6.3 Hz, 1H), 4.83 (dd, *J* = 13.6, 6.8 Hz, 2H), 4.63 (s, 2H), 4.40 (dd, *J* = 11.2, 4.3 Hz, 1H), 4.27 (ddd, *J* = 12.6, 9.4, 3.9 Hz, 1H), 4.21 (ddd, *J* = 11.2, 8.3, 4.8 Hz,

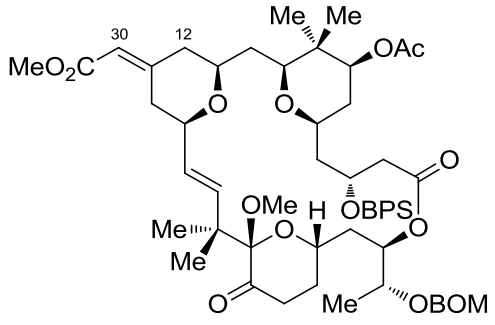
1H), 4.05 (dd, *J* = 14.6, 6.3 Hz, 1H), 3.89-3.85 (m, 1H), 3.66 (q, *J* = 5.8 Hz, 2H), 3.33 (s, 3H), 2.95-2.91 (m, 1H), 2.71-2.67 (m, 2H), 2.62-2.51 (m, 3H), 2.41-2.31 (m, 4H), 2.10 (dd, *J* = 14.1, 11.7 Hz, 1H), 2.02 (s, 3H), 1.94-1.83 (m, 4H), 1.74-1.64 (m, 4H), 1.57 (dd, *J* = 12.2, 9.2 Hz, 1H), 1.53 (t, *J* = 3.4 Hz, 1H), 1.50 (t, *J* = 3.4 Hz, 1H), 1.40-1.33 (m, 2H), 1.24 (d, *J* = 6.3 Hz, 3H), 1.20 (s, 3H), 1.07 (s, 3H), 1.03 (s, 9H), 0.81 (s, 3H), 0.71 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 206.7, 206.5, 173.4, 170.6, 139.2, 137.5, 136.0(x2), 133.9, 133.4, 130.1, 130.0, 128.7, 128.0(x2), 127.9(x2), 127.0, 94.0, 79.8, 78.2(x2), 76.8, 74.7, 73.3, 71.2, 70.1, 69.7, 69.2, 51.9, 48.2, 47.2, 44.0, 43.6, 43.2, 39.1, 37.4, 37.3, 36.4, 33.8, 30.4, 27.0, 23.2, 22.3(x2), 21.3, 19.4, 17.0, 13.6; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 51.9, 27.0, 23.2, 22.3(x2), 21.3, 17.0, 13.6; CH_2 δ 94.0, 70.0, 48.1, 47.2, 43.6, 43.2, 39.1, 37.3, 36.4, 33.8, 30.4; CH_1 δ 139.2, 136.0, 130.1, 130.0, 128.6, 128.0(x2), 127.9(x2), 127.0, 79.8, 78.2, 78.1, 76.8, 74.7, 73.3, 71.2, 69.7, 69.2; CH_0 δ 206.8, 206.5, 173.4, 170.6, 137.5, 126.0, 133.9, 133.4, 44.0, 37.4, 19.4; IR (neat) 2970, 2935, 1732, 1607, 1513, 1455, 1377, 1247, 1170, 1103, 1036, 823, 741, 700 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{58}H_{80}NaO_{14}Si$ (M+Na): 1051.5215, found: 1051.5232.



Preparation of (1*S*,3*R*,7*R*,11*S*,15*S*,17*R*,21*R*,23*S*,25*S*,*E*)-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-21-((*tert*-butyl diphenylsilyl)oxy)-11-methoxy-10,10,26,26-tetramethyl-5,12,19-trioxo-18,27,28,29-tetraoxatetracyclo [21.3.1.13, 7.111,15] nonacos-8-en-25-yl acetate (1.137): To a

stirring solution of the seco acid **1.141** (29 mg, 0.028 mmol, 1.0 equiv) in THF (1 mL) in a 5 mL vial at 0 °C was added triethylamine (23 µL, 0.17 mmol, 6.0 equiv) and 2,4,6-trichlorobenzoyl chloride (13 µL, 0.086 mmol, 3.0 equiv). After 1 h, the reaction was warmed to rt and stirring continued for an additional 5 h. The reaction mixture was diluted with 3:1 toluene/ THF (10 mL) and taken up in a 25 mL gas-tight syringe. This solution was added by syringe pump to a stirring solution of DMAP (70 mg, 0.57 mmol, 20.0 equiv) in toluene (20 mL) at 45 °C over 12 h. The residual contents of the syringe were rinsed into the flask with toluene (0.5 mL) and stirring continued for an additional 2 h. The reaction mixture was cooled to rt and washed with saturated aqueous $NaHCO_3$ solution (2 x 10 mL) and brine (2 x 10 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 1 x 10 cm silica gel column, eluting with 20% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (15-30) were combined and concentrated under reduced pressure to provide pure macrolactone **1.137** as a white foam (23 mg, 79%): R_f = 0.5 (40% EtOAc/hexanes); $[\alpha]_D^{20} = +3.7$ (*c* = 1.5, CHCl3); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.71-7.69 (m, 5H), 7.47-7.30 (m, 10H), 6.31 (d, *J* = 15.6 Hz, 1H), 5.50 (ddd, *J* = 11.2, 3.9, 2.4 Hz, 1H), 5.38 (dd, *J* = 16.1, 8.3 Hz, 1H), 4.87 (d, *J* = 6.8 Hz, 1H), 4.81 (d, *J* = 6.8 Hz, 1H), 4.65 (s, 2H), 4.43 (ddd, *J* = 10.3, 7.4, 4.5 Hz, 2H), 4.23-4.18 (m, 2H), 4.16-4.12 (m, 1H), 4.04 (ddd, *J* = 10.7, 7.8, 4.3 Hz, 1H), 3.54-3.50 (m, 1H), 3.24 (s, 3H), 2.68 (ddd, *J* = 14.6, 9.7, 5.3 Hz, 1H), 2.58 (dd, *J* = 15.1, 9.2 Hz, 1H), 2.47-2.29 (m, 8H), 2.18-2.13 (m, 1H), 2.12-2.03 (m, 1H), 1.99 (s, 3H), 1.97-1.85 (m, 3H), 1.58-1.35 (m, 4H), 1.33 (s, 3H), 1.17 (d, *J* = 6.3 Hz, 3H), 1.12 (ddd, *J* = 12.2, 5.2, 2.8 Hz, 1H), 1.00 (s, 3H), 0.98 (s, 9H), 0.73 (s, 3H), 0.65 (s, 3H); 125 MHz

^{13}C NMR ($CDCl_3$) δ 206.8, 205.4, 171.7, 170.6, 142.2, 137.9, 136.2, 135.9, 134.5, 133.3, 130.3, 129.9, 128.6, 128.1, 128.0, 127.8 (x2), 126.0, 93.7, 80.5, 79.0, 76.9, 74.4, 74.1, 72.2, 70.8, 70.7, 69.7, 65.2, 53.5, 48.4, 47.8, 44.9, 43.6, 43.5, 37.9, 37.2, 35.9, 33.4, 33.3, 31.1 29.9, 27.0, 24.7, 22.2, 21.2, 19.7, 19.5, 14.9, 13.5; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 52.5, 27.0, 24.7, 22.2, 21.2, 19.7, 14.9, 13.5; CH_2 δ 93.7, 69.7, 48.4, 47.8, 44.9, 43.9, 37.9, 35.9, 33.4, 33.3, 31.1; CH_1 δ 142.2, 136.1, 135.9, 130.3, 129.9, 128.6, 128.0 (x2), 127.8, 126.0, 80.5, 79.0, 77.4, 76.8, 74.3, 74.1, 72.2, 70.7, 65.2; CH_0 δ 206.8, 205.4, 171.7, 170.6, 137.9, 134.5, 133.3, 127.8, 70.8, 43.5, 37.2, 29.9, 19.5; IR (neat) 2933, 2857, 2337, 1733, 1652, 1540, 1456, 1363, 1150, 1109, 1038, 747, 703 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{58}H_{78}NaO_{13}Si$ (M+Na): 1033.5109, found: 1033.5096.

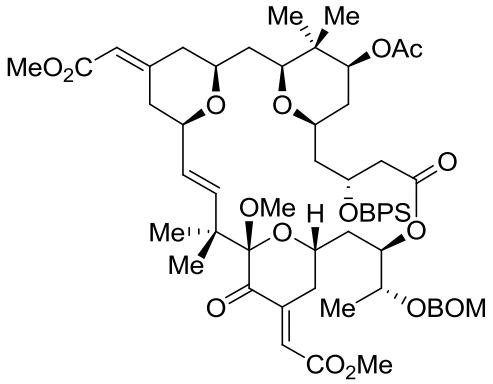


Preparation of (*Z*)-methyl 2-((1*S*,3*S*,7*R*, 11*S*,15*S*, 17*R*,21*R*,23*S*,25*S*,*E*) -25-acetoxy-17-((*R*)-1-((benzyloxy) methoxy)ethyl)-21-((*tert*-butyldiphenylsilyl)oxy)-11-methoxy-10,10,26,26-tetra methyl -12,19-dioxo-18,27,28,29-tetraoxatetracyclo [21.3.1.13,7.111,15]nonacos-8-en-5-ylidene)acetate (1.139): To a stirring solution of the *R*-BINOL phosphonate **1.138** (58 mg, 0.144 mmol, 8 equiv) in THF (1.5 mL) in a 5 mL vial at -78 °C was added a 1M solution of NaHMDS in THF (135 µL, 0.135 mmol, 7.5 equiv) down the wall of the vial via syringe. The resulting solution was stirred at -78 °C for 30 min. During this time, the diketone **1.137** (18.3 mg, 0.018 mmol, 1equiv) was dissolved in 0.1 mL of THF and added dropwise along the side of the vial via syringe and then rinsed in with THF (2 x 0.1

mL). The solution was stirred at -78 °C for 1 h then warmed to 0 °C and stirring was continued for 12 h. The reaction was quenched by dropwise addition of saturated aq. NH_4Cl solution (3 mL) and was diluted with EtOAc (3 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 5 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. Purification was accomplished using flash column chromatography with a 2 x 7 cm silica gel column, eluting with 15% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (34-45) were combined and concentrated under reduced pressure to provide the desired unsaturated ester as a 4:1, *Z*:*E* mixture of diastereomers. The *E* and *Z* diastereomers were further separated using preparative thin layer chromatography eluting with 10% EtOAc/benzene providing 13 mg of the desired *Z* isomer as 12:1 mixture and 3.3 mg of the *E* isomer (combined yield 85%): The stereochemistry of *Z* isomer was confirmed by a NOE interaction between the C_{31} olefin proton and the C_{12} equatorial proton. R_f = 0.55 (30% EtOAc/hexanes); Analytical data for **Z** isomer: $[\alpha]_D^{20} = +12.8$ (*c* = 1.0, CHCl3); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.68 (m, 4H), 7.45-7.28 (m, 11H), 6.26 (d, *J* = 15.6 Hz, 1H), 5.78 (s, 1H), 5.44-5.39 (m, 2H), 4.91(d, *J* = 6.8 Hz, 1H), 4.83 (d, *J* = 6.8 Hz, 1H), 4.68 (d, *J* = 12.2 Hz, 1H), 4.62 (d, *J* = 12.2 Hz, 1H), 4.44 (ddd, *J* = 12.8, 4.0, 4.0 Hz, 1H), 4.27 (dd, *J* = 11.3, 2.8 Hz, 1H), 4.18-4.14 (m, 1H), 4.02 (ddd, *J* = 15.1, 7.5, 3.9 Hz, 1H), 3.94-3.90 (m, 1H), 3.84-3.76 (m, 1H), 3.73 (s, 3H), 3.38-3.34 (m, 1H), 3.23 (s, 3H), 2.64 (ddd, *J* = 14.1, 9.2, 5.3 Hz, 1H), 2.57-2.44 (m, 3H), 2.40-2.34 (m, 1H), 2.23-2.18 (m, 1H), 2.15-2.2.08 (m, 1H), 2.04-1.99 (m, 3H), 2.0 (s, 3H), 1.91-1.80 (m, 2H), 1.51-1.44 (m, 2H), 1.41-1.37 (m, 2H), 1.32 (s, 3H), 1.26 (d, *J* = 2.4 Hz, 1H), 1.18 (d, *J* = 6.3 Hz, 3H), 1.12-1.09 (m, 1H), 1.04-1.02 (m, 1H), 1.00 (s, 3H), 0.98 (s, 9H), 0.73 (s, 3H),

0.68 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 205.7, 171.4, 170.6, 167.0, 157.2, 141.5, 136.1, 136.0, 135.9, 134.6, 133.5, 130.0, 129.8, 128.5, 128.0, 127.9, 127.8, 127.7, 120.0, 114.9, 93.8, 81.0, 79.6, 76.8, 75.5, 74.0, 72.3, 70.9, 70.8, 69.6, 65.8, 52.5, 51.2, 44.8, 43.6, 43.5, 43.1, 43.0, 37.9, 37.3, 36.0, 35.8, 33.4(x2), 30.8, 27.0, 24.6, 22.3, 21.2, 19.8, 19.4, 15.0, 13.5; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 52.5, 51.2, 27.0, 24.6, 22.3, 21.3, 19.8, 15.1, 13.5; CH_2 δ 93.8, 69.6, 44.0, 43.6, 43.0, 37.9, 36.0, 35.8, 33.4(x2), 30.8; CH_1 δ 141.6, 136.1, 135.9, 130.0, 129.9, 128.5, 128.0, 127.9, 127.8, 127.7, 127.0, 114.9, 81.0, 79.6, 76.8, 75.5, 74.0, 72.3, 70.9, 70.8; CH_0 δ 205.7, 171.4, 170.6, 167.0, 157.2, 136.0, 134.6, 65.8, 43.1, 37.3, 30.8, 19.4; IR (neat) 2934, 2361, 2337, 1734, 1652, 1456, 1374, 1243, 1149, 1107, 1028 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{61}H_{82}NaO_{14}Si$ (M+Na): 1089.5372, found: 1089.5371. Spectroscopic data for ***E*** isomer: 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.67 (m, 4H), 7.45-7.30 (m, 11H), 6.27 (d, *J* = 15.6 Hz, 1H), 5.77 (s, 1H), 5.49 (ddd, *J* = 11.8, 3.9 1.8 Hz, 1H), 5.38 (dd, *J* = 15.6, 8.3 Hz, 1H), 4.89 (d, *J* = 6.8 Hz, 1H), 4.81 (d, *J* = 6.6 Hz, 1H), 4.66 (s, 2H), 4.45 (ddd, *J* = 11.6, 6.0, 2.0 Hz, 1H), 4.23 (dd, *J* = 11.2, 6.3 Hz, 1H), 4.19 (dd, *J* = 10.7, 3.9 Hz, 1H), 4.04-3.98 (m, 2H), 3.93 (d, *J* = 13.6, 1H), 3.78 (s, 3H), 3.29 (t, *J* = 10.7, 1H), 3.24 (s, 3H), 2.68 (ddd, *J* = 19.5, 9.2, 5.3 Hz, 1H), 2.58-2.45 (m, 3H), 2.37 (ddd, *J* = 20.6, 8.0, 5.8 Hz, 1H), 2.27-2.23 (m, 1H), 2.21-2.13 (m, 3H), 1.99 (s, 3H), 1.93-1.83 (m, 3H), 1.77-1.72 (m, 3H), 1.52 (dd, *J* = 13.6, 7.8 Hz, 2H), 1.47-1.39 (m, 2H), 1.32 (s, 3H), 1.18 (d, *J* = 6.3 Hz, 3H), 1.06-1.04 (m, 1H), 1.01 (s, 3H), 0.98 (s, 9H), 0.71 (s, 3H), 0.69 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 205.6, 171.6, 170.5, 167.1, 157.7, 141.7, 137.9, 136.1, 135.9, 134.6, 133.5, 130.2, 129.9, 128.6, 128.0, 127.8, 126.9, 114.7, 93.6, 81.1, 80.2, 77.4, 76.9, 75.4, 73.9, 72.1, 70.9, 70.7, 69.7, 65.4, 52.6, 51.2, 44.9, 43.6, 42.6, 37.9, 37.3, 37.3, 36.3, 36.0, 33.4,

33.2, 31.2, 29.9, 27.0, 24.8, 22.3, 21.3, 19.8, 19.5, 14.8, 13.6; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 52.6, 51.2, 27.0, 24.8, 22.3, 21.3, 19.8, 14.8, 13.6; CH_2 δ 93.6, 69.7, 44.9, 43.6, 42.6, 37.9, 36.3, 36.0, 33.4, 33.2, 31.2, 29.9; CH_1 δ 141.7, 136.1, 135.9, 130.3, 129.9, 128.6, 128.1, 128.0, 127.8, 126.9, 114.7, 81.1, 80.2, 77.4, 76.9, 75.4, 73.9, 72.1, 70.9, 70.7, 65.4; CH_0 δ 205.6, 171.6, 170.5, 167.1, 157.7, 137.9, 134.6, 133.5, 37.3, 19.5.

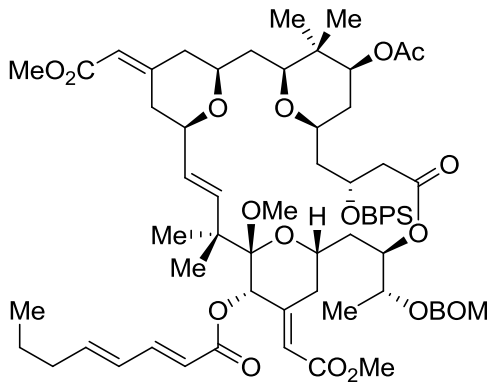


Preparation of (2*Z*,2'*E*)-dimethyl 2,2'-((1*S*,3*S*,7*R*,11*S*,15S,17*R*,21*R*,23*S*,25*S*,*E*)-25-acetoxy-17-((*R*)-1-((benzyloxy) methoxy)ethyl)-21-((tert-butyldiphenylsilyl)oxy)-11-methoxy-10,10,26,26-tetramethyl-12,19-dioxo-18,27,28,29-tetraoxatetracyclo [21.3.1.13,7.111,15]nonacos-8-ene-5,13-diylidene)diacetate (1.146): To a stirring solution of ketone **1.139-*Z*** (5.0 mg, 0.00468 mmol, 1.0 equiv) in 234 µL of MeOH in a 4 mL vial at room temperature was added K_2CO_3 (3.2 mg, 0.0234 mmol, 5 equiv) and the methyl acetal of methyl glyoxylate (11 mg, 0.0936 mmol, 20 equiv). The mixture was stirred overnight, during which time the color of the solution changed to yellow. The reaction was quenched by addition of 3 mL of saturated aqueous NH_4Cl solution and was then diluted with 5 mL of EtOAc. The phases were separated and the aqueous phase was extracted with EtOAc (3 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under

reduced pressure. This crude material was taken into the following reaction without further purification.

To a stirring solution of the aforementioned product in pyridine (500 µL, 0.01 M) in a 4 mL reaction vial at rt was added DMAP (6 mg, 0.0468 mmol, 10 equiv), and acetic anhydride (22 µL, 0.234 mmol, 50 equiv). The reaction mixture was allowed to stir at rt for 24 h and was then quenched by addition of saturated aqueous $NaHCO_3$ solution (4 mL). The mixture was diluted with 5 mL CH_2Cl_2, the phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 1 x 5 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 4 ml fractions. The product containing fractions (9-21) were combined and concentrated under reduced pressure to provide pure enoate **1.146** (2.3 mg, 60% over 2 steps): R_f = 0.44 (40% EtOAc/hexanes); $[\alpha]_D^{20} = +8$ (*c* = 0.4, CHCl3); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.73-7.70 (m, 4H), 7.45-7.27 (m, 11H), 6.56 (dd, *J* = 2.4, 1.9 Hz, 1H), 5.97 (d, *J* = 15.6 Hz, 1H), 5.72 (s, 1H), 5.42 (dd, *J* = 15.6, 6.8 Hz, 1H), 5.22-5.20 (m, 1H), 4.84 (dd, *J* = 16.1, 6.8 Hz, 2H), 4.65 (s, 2H), 4.38 (dd, *J* = 11.2, 4.3 Hz, 1H), 4.34-4.33 (m, 1H), 4.09-4.05 (m, 2H), 3.72 (s, 3H), 3.70 (s, 3H), 3.46-3.41 (m, 2H), 3.24 (s, 3H), 2.96 (dd, *J* = 10.7, 2.9 Hz, 1H), 2.92 (dd, *J* = 10.7, 3.4 Hz, 1H), 2.68 (dd, *J* = 15.1, 6.8 Hz, 2H), 2.62-2.56 (m, 2H), 2.22 (ddd, *J* = 14.9, 6.61, 2.7 Hz, 1H), 2.15-2.03 (m, 2H), 2.00 (s, 3H), 1.89 (t, *J* = 13.4 Hz, 1H), 1.75-1.69 (m, 1H), 1.58-1.49 (m, 2H), 1.47 (t, *J* = 4.8 Hz, 1H), 1.44 (t, *J* = 4.3 Hz, 1H), 1.26 (s, 3H), 1.25(s, 3H), 1.23 (d, *J* = 2.9 Hz, 3H), 0.99 (s, 9H), 0.96 (s, 3H), 0.76 (s, 3H), 0.67 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 220.5, 171.0. 170.5, 166.9, 166.1, 157.2, 147.9,

137.9, 136.1, 136.0, 134.5, 133.7, 129.9, 129.8, 129.2, 128.5, 127.9, 127.8 (x2), 123.1, 114.8, 103.5, 93.8, 80.9, 78.6, 77.4, 77.0, 76.2, 73.2, 73.0, 71.9, 69.7, 63.3, 52.4, 52.0, 51.2, 44.4, 43.2, 43.1, 37.8, 37.9, 36.8, 36.3, 34.5, 34.3, 33.3, 29.9, 27.0, 24.8, 23.6, 22.4, 21.3, 21.1, 19.4, 15.4, 13.6; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 52.4, 52.0, 51.2, 27.0, 22.4, 22.3, 21.3, 21.1, 15.4, 13.6; CH_2 δ 103.5, 93.8, 69.7, 43.1, 37.8, 36.8, 36.2, 34.5, 34.3, 33.3, 24.8; CH_1 δ 136.1, 136.0, 129.9, 129.8, 129.2, 128.5, 127.9, 127.8(x), 123.1, 114.7, 80.9, 78.6, 77.4, 77.0, 76.2, 73.2, 73.0, 71.9, 69.2; CH_0 δ 220.5, 171.0, 170.5, 166.9, 166.1, 157.2, 147.9, 137.9, 134.5, 133.7, 63.3, 37.9, 23.6, 19.4; IR (neat) 2952, 2359, 2341, 1662, 1616, 1586, 1513, 1454, 1381, 1300, 1248, 1172, 1092, 1038, 848, 736, 698 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{64}H_{84}NaO_{16}Si$ (M+Na): 1159.5426, found: 1159.5419.

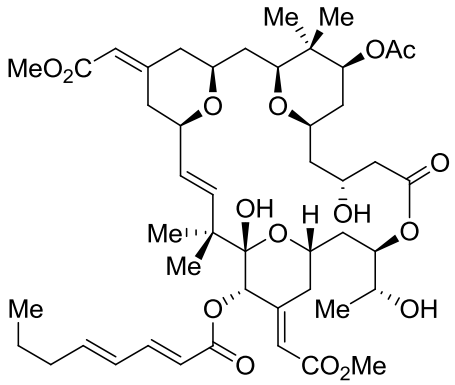


Preparation of (2*Z*,2'*E*)-dimethyl 2,2'-((1*S*,3*S*,7*R*,11*S*,12*S*,15*S*,17*R*,21*R*,23*S*,25*S*,*E*)-25-acetoxy-17-((*R*)-1-((benzyloxy) methoxy)ethyl)-21-((*tert*-butyldiphenylsilyl)oxy)-11-methoxy-10,10,26,26-tetramethyl-12-((2*E*,4*E*)-octa-2,4-dienoyloxy)-19-oxo-18,27,28,29-tetraoxatetracyclo[21.3.1.13,7.111,15]nonacos-8-ene-5,13-diylidene)diacetate (1.147): To a stirring solution of ketone **1.146** (4.1 mg, 0.0036 mmol, 1.0 equiv) in MeOH (360 µL, 0.01 M) in a 5 mL reaction vial at rt was added $CeCl_3 \cdot 7H_2O$ (27 mg, 0.072 mmol, 20.0 equiv). The mixture was stirred until all the $CeCl_3 \cdot 7H_2O$ was completely dissolved.

The mixture was then cooled to -42 ºC and stirred for 10 min and then $NaBH_4$ (1.3 mg, 0.036 mmol, 10.0 equiv) was added. Stirring continued for 1 h at -42 ºC after which the reaction was quenched by slow addition of saturated aqueous NH_4Cl solution (2 mL) and diluted with 40% EtOAc/hexanes (5 mL). The layers were separated and the aqueous layer was extracted with 40% EtOAc/hexanes (3 x 5 mL). The organic phase was washed with brine (5 mL), then dried over Na_2SO_4, filtered and concentrated under reduced pressure to provide the crude intermediate alcohol which was carried directly to the next step without purification.

To a stirring solution of this alcohol in CH_2Cl_2 (360 µL, 0.001 M) in a 5 mL reaction vial at rt was added pyridine (15 µL, 0.18 mmol, 50 equiv), DMAP (4.0 mg, 0.036 mmol, 10 equiv), and octadienoic anhydride (29 mg, 0.108 mmol, 30 equiv). The reaction mixture stirred at rt for 12 h and was then quenched by the addition of saturated aqueous $NaHCO_3$ solution (2.0 mL). The mixture was stirred vigorously for 30 min and was then diluted with 40% EtOAc/hexanes (5mL). The phases were separated and the aqueous phase was extracted with 40% EtOAc/hexanes (3 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography using 10% EtOAc/hexanes (50 mL) followed by 20% EtOAc/hexanes collecting 4 mL fractions. Fractions 16-23 provided the desired product as 4.2:1 mixture of diastereomers which were further separated by a preparative TLC with 4% acetone/benzene to provide the ester **1.147** (3 mg, 66%, 2 steps) as a pale yellow liquid. R_f = 0.53 (30% EtOAc/hexanes); $[\alpha]_D^{20} = +12$ (*c* = 0.11, CHCl3); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.71-7.69 (m, 4H), 7.45-7.24 (m, 11H), 6.20-6.15(m, 2H), 5.99 (d, *J* = 1.6 Hz, 1H), 5.79-5.76 (m, 2H), 5.60 (ddd,

J = 7.2, 5.6, 3.9 Hz, 1H), 5.31(dd, *J* = 15.6, 8.7 Hz, 1H), 5.22 (s, 1H), 4.94 (d, *J* = 6.8 Hz, 1H), 4.84 (d, *J* = 6.8 Hz, 1H), 4.71 (d, *J* = 12.2 Hz, 1H), 4.67 (d, *J* = 12.2 Hz, 1H), 4.44 (ddd, *J* = 13.2, 6.3, 3.5 Hz, 1H), 4.24 (dd, *J* = 11.2, 4.8 Hz, 1H), 4.18-4.13 (m, 1H), 3.90-3.81 (m, 2H), 3.75 (s, 3H), 3.69 (s, 3H), 3.28 (t, *J* = 10.7, 1H), 3.05 (s, 3H), 2.51 (dd, *J* = 15.1, 9.7 Hz, 1H), 2.35-2.34 (m, 3H), 2.22-2.13 (m, 4H), 1.98 (s, 3H), 1.49-1.44 (m, 4H), 1.40-1.26 (m, 7H), 1.18 (d, *J* = 6.3, 1H), 1.10 (s, 3H), 1.06 (s, 3H), 1.05-1.00 (m, 2H), 0.97 (s, 12H), 0.92(t, *J* = 7.3, 3H), 0.69 (s, 3H), 0.66 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 171.7, 170.6, 167.0, 165.6, 157.2, 151.7, 146.7, 145.9, 142.9, 138.0, 136.2, 135.9, 134.7, 133.5, 130.0, 129.8, 128.1, 127.9, 127.8, 125.1, 121.0, 119.4, 118.6, 115.0, 103.4, 93.8, 81.0, 80.3, 76.8, 75.8, 74.1, 73.3, 72.5, 70.8, 70.1, 69.6, 67.2, 52.9, 51.3, 51.2, 45.4, 45.2, 43.8, 43.0, 38.0, 37.2, 36.8, 35.8, 35.3, 34.5, 33.7, 33.6, 31.1, 27.0, 25.8, 22.3, 22.0, 21.3, 20.1, 19.4, 15.0, 13.9, 13.4; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 52.9, 51.3, 51.2, 27.0, 25.8, 22.3, 21.3, 20.1, 15.0, 13.9, 13.4; CH_2 δ 93.8, 69.7, 45.2, 43.8, 430, 38.0, 36.8, 35.8, 35.2, 33.6, 31.1, 22.0; CH_1 δ 146.7, 145.9, 142.9, 136.2, 135.9, 130.0, 129.8, 128.5, 128.1, 127.9, 127.8, 125.1, 119.4, 118.6, 115.4, 81.0, 80.3, 77.4, 76.8, 75.8, 74.1, 73.7, 72.5, 70.8, 70.1, 67.2; CH_0 δ 171.7, 170.6, 167.0, 165.6, 157.2, 151.7, 138.0, 134.7, 121.0, 103.4, 45.4, 37.2, 34.5, 19.4; IR (neat) 2993, 1738, 1680, 1513, 1462, 1383, 1364, 1247, 1110, 1079, 1040, 821, 736, 701, 665 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{72}H_{96}NaO_{17}Si$ (M+Na): 1283.6315, found: 1283.6298.



Preparation of C_9-Deoxy Bryostatin 1 (Merle 30): To a stirring solution of the BPS ether **1** (2.3 mg, 0.0018 mmol, 1.0 equiv) in a 5:4:1 THF/MeOH/ pyridine solution (0.9 mL, 0.002M) at 0 ºC in a 2 mL plastic centrifuge tube was added HF·Py (20 %, 0.40 mL). The reaction mixture was stirred at 0 ºC for 30 min and then warmed to rt. Stirring was continued for 72 h and the reaction mixture was then quenched by pipetting into a mixture of saturated aqueous $NaHCO_3$ solution and EtOAc (5 mL each). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 5 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was taken to the next step without further purification.

To a 4 mL reaction vial containing the analogue precursor from the previous reaction was added a 0.25 M solution of $LiBF_4$ in 25:1 CH_3CN/ H_2O (328 µL, 0.082 mmol, 45.0 equiv). The reaction vial was sealed and the mixture was allowed to stir at 80 ºC for 14 h. After cooling to rt, the reaction mixture was diluted with EtOAc (6 mL) and was quenched with saturated aqueous $NaHCO_3$ solution (5 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated. Purification was accomplished using flash column chromatography with a 0.5 x 6 cm silica gel column, eluting with 20% EtOAc/hexanes, collecting 6 x 50 mm test tube fractions (1-10)

followed by 50% EtOAc/hexanes. The product containing fractions (11-45) were combined and concentrated under reduced pressure to provide C9-deoxy bryostatin 1 (1.1 mg, 68% over 2 steps) as white solid: R_f = 0.3 (50% EtOAc/hexanes; $[\alpha]_D^{20} = +5$ (*c* = 0.11, EtOAc); 500 MHz ^{1}H NMR ($CDCl_3$) δ 6.18-6.17 (m, 1H), 6.01 (d, *J* = 1.5 Hz, 1H), 5.8 (dd, *J* = 15.1, 6.8 Hz, 2H), 5.68 (s, 1H), 5.33 (dd, *J* = 15.6, 8.3 Hz, 1H), 5.24-5.18 (m, 3H), 4.58 (dd, *J* = 11.2, 4.3 Hz, 1H), 4.37 (d, *J* = 12.2 Hz, 1H), 4.25-4.21 (m, 1H), 4.09-4.01 (m, 2H), 3.81 (dd, *J* = 12.2, 6.3 Hz, 1H), 3.72 (s, 3H), 3.67 (s, 3H), 3.59-3.56 (m, 2H), 3.17 (dd, *J* = 11.7, 1.99 Hz, 1H), 2.48 (dd, *J* = 12.9, 2.7 Hz, 1H), 2.40 (t, *J* = 11.7 Hz, 1H), 2.21-2.07 (m 6H), 2.06 (s, 3H), 2.00-1.78 (m, 6H), 1.73 (ddd, *J* = 12.5, 4.4, 1.8 Hz, 2H), 1.53-1.45 (m, 7H), 1.25 (d, *J* = 6.3 Hz, 3H), 1.16 (s, 3H), 1.01 (s, 3H), 0.93 (t, *J* = 7.3 Hz, 3H), 0.91 (s, 3H), 0.83 (s, 3H). ; 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.0, 170.9, 167.2, 166.9, 165.7, 165.2, 156.7, 152.1, 151.0, 146.5, 145.6, 139.1, 129.8, 128.5, 121.2, 119.7, 118.8, 114.5, 99.2, 85.0, 79.0, 76.7, 74.2, 73.7, 70.4, 68.8, 64.8, 51.2(x2), 45.1, 44.2, 42.9, 40.1, 37.7, 36.6, 36.2, 36.0, 35.2, 33.9, 31.5, 24.8, 22.7, 22.1, 21.3, 20.1, 20.0, 13.9; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 51.2 (x2), 24.8, 22.7, 21.3, 20.0 (x2), 13.9; CH_2 δ 44.2, 43.0, 40.1, 36.6, 36.3, 36.1, 35.2, 34.0, 31.5, 22.1; CH δ 146.5, 145.6, 139.2, 129.8, 128.6, 119.8, 118.9, 114.6, 85.1, 79.0, 76.7, 74.3, 73.8, 70.4, 68.8, 64.9; CH_0 δ 172.4, 170.9, 167.2, 166.9, 165.7, 156.7, 152.1, 121.2, 45.1, 37.7; IR (neat) 2360, 2341, 1732, 1470, 1428, 1263, 1245, 1166, 1110, 1037 cm^{-1}; LRMS calcd for $C_{47}H_{68}O_{16}$ (M+Na): 911.4405, found: 911.4417.

Biological Experiments and Data for Merle 30

[^{3}H]PDBu binding assay: The binding assay for Merle 30 was performed similar to that of Merle 28. The K_i for Merle 30 was found to be 0.38 ± 0.07 nM (average of three determinations).

Attachment and cell proliferation of U937 cells: The attachment and proliferation assay for Merle 30 was done similar to that of Merle 28 and the results are shown in Figure **1.69** and Figure **1.70**.

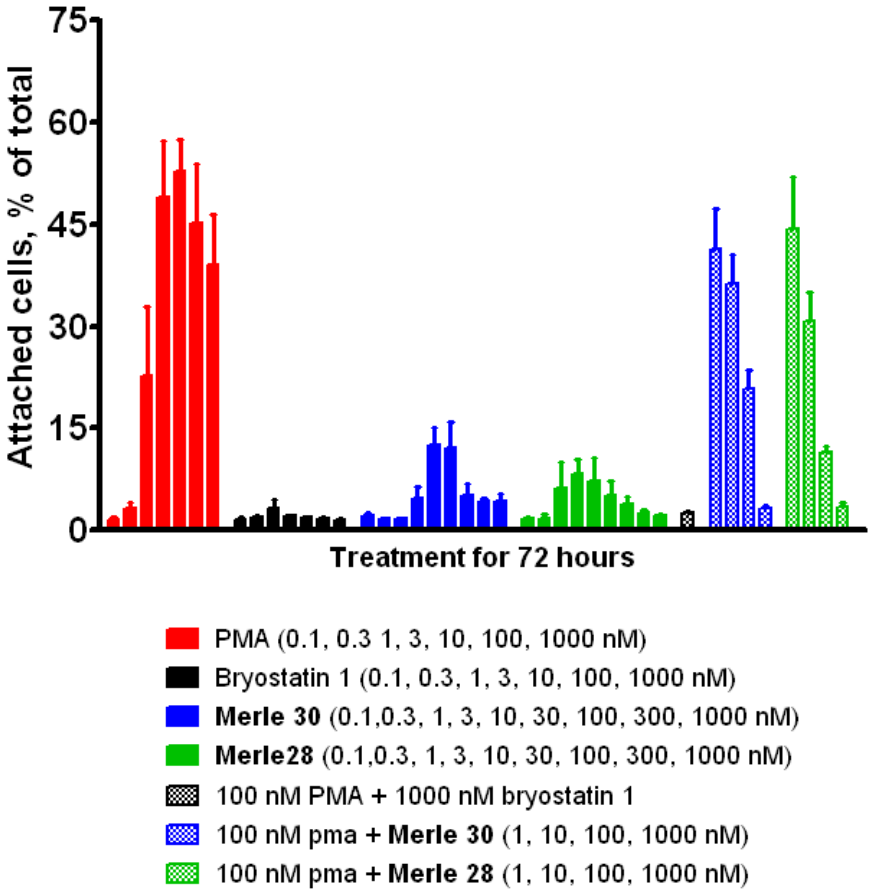


Figure 1.69. Attachment Assay for Merle 28 and Merle 30

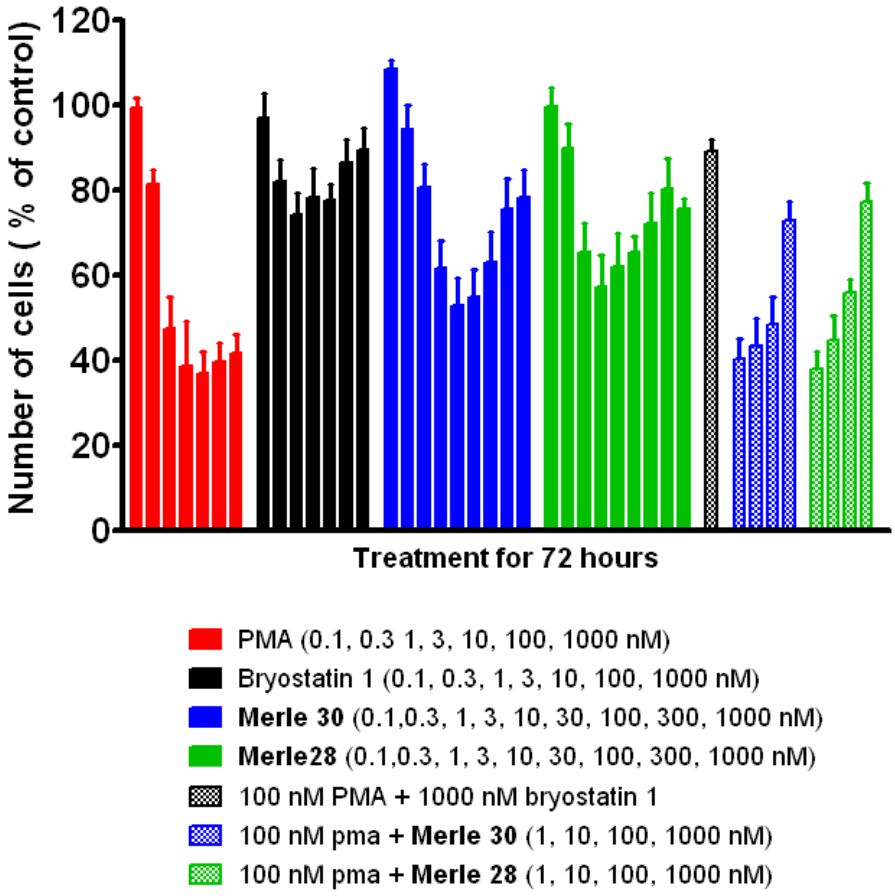


Figure 1.70. Inhibition of Proliferation Assay for Merle 28 and Merle 30

Proliferation of LNCaP cells (Figure **1.71**): LNCaP human prostate cancer cells (from ATCC, Manassas, VA) were plated at a concentration of 80,000 cells/ml (1 ml total volume) into the wells of 24 well plates and cultured for 2 days in RPMI-1640 medium containing 10 % fetal bovine serum (ATCC, Manassas, VA). The cells were then treated with the indicated concentrations of the experimental compounds diluted in DMSO (final DMSO concentration 0.1 %). The LNCaP cells were imaged by phase contrast microscopy automatically every 2 h using an Incucyte instrument (Essen

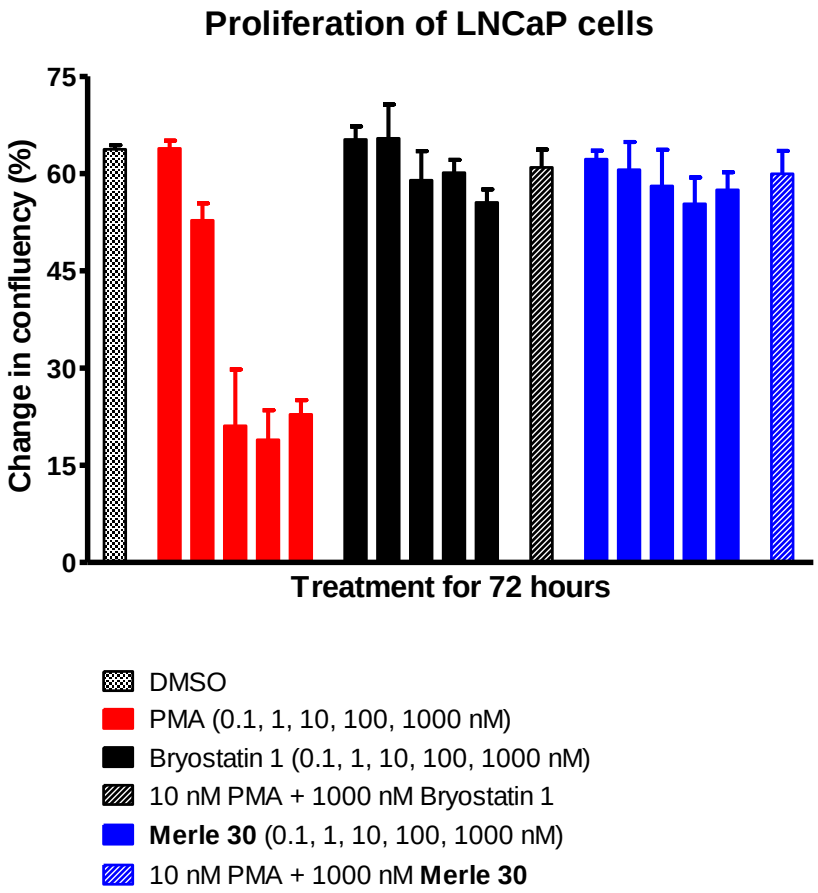


Figure 1.71. Proliferation of LNCaP Cells by Merle 30

Instruments, Ann Arbor, MI) starting 24 h before compound treatment and continuing for 72 h after treatment. The proliferation of the LNCaP cells was expressed as the difference in cell confluency before and after treatment as determined by the instrument. Three independent experiments were performed and values represent the mean ± SEM of the three experiments.

Secretion of TNF-alpha from LNCaP cells (Figure **1.72**): 200,000 LNCaP cells in 1 ml medium were plated into the wells of 24 well plates and cultured for 2 days in RPMI-1640 medium containing 10 % fetal bovine serum (ATCC, Manassas, VA). The

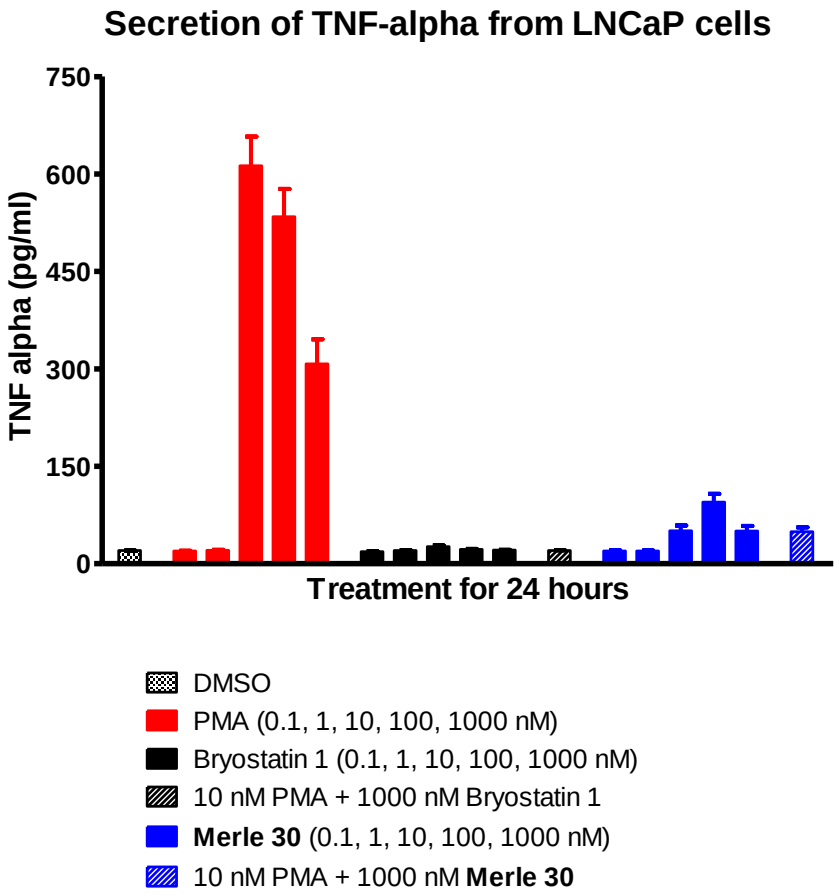


Figure 1.72. The Effect of Merle 30 Compared to That of PMA and Bryostatin 1 on the Secretion of Tumor Necrosis Factor α by LNCaP Cells

cells were then treated with the indicated concentrations of the experimental compounds diluted in DMSO (final DMSO concentration 0.1 %). The medium was collected 24 h after treatment and centrifuged at 1500 x g to remove floating cells. The amount of TNF-alpha secreted into the medium was then measured by ELISA (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Four independent experiments were performed and values represent the mean ± SEM of the four experiments.

Description of Modeling and Docking Approach for Merle 30

Before beginning the docking we first performed a conformational search of bryostatin 1 in implicit water and octanol solvents. The global energy minimum conformation found in both solvents was essentially identical to the crystal and NMR conformations, and characterized by a bifurcated H-bond between the proton of the C3-OH and the pyran oxygens of the A- and B-rings, and a second H bond between the proton of the C19-OH and the oxygen of the C3-OH. The four lowest-energy conformations from the search were saved, and corresponding structures of C9-deoxy bryostatin 1 for each of these conformations were built by simply replacing the C9 hydroxyl with a hydrogen atom.

These conformations of bryostatin 1 and C9-deoxy bryostatin were then docked into the crystal structure of the C1b domain of PKC-δ.[77] We included a similarity constraint to the crystallized phorbol-13-acetate ligand to bias the optimization toward solutions where the acceptor atoms in bryostatin 1 and phorbol are close in space. The highest-scoring pose for both compounds was the global minimum conformation in solution, suggesting that bryostatin 1 does not undergo a conformational change upon binding to the C1 domain.

Modeling methods: First, a conformational search of the bryostatin 1 was performed. The initial structure for bryostatin 1 was downloaded from the Cambridge Structural Database (reference code BOKKIV). Hydrogen atoms were added as needed to fill valences. Two conformational searches were performed, using the Monte Carlo multiple minimum (MCMM) method in the MacroModel program from Schrödinger.[78] Both searches used the OPLS_2005 forcefield, one with water implicit solvent, and the other

with octanol, to see whether the polarity of the environment had any effect on bryostatin's conformational preferences. During the conformational searches all torsions were varied for 5,000 MCMM steps, but all of the chiral centers and double bonds were restricted to the crystal conformation. After each step the resulting structure was energy minimized to a gradient convergence of 0.05. If the minimized structure was within an energy cutoff of 41 kJ/mol of the global minimum, it was then compared to previously stored structures and either kept as a unique conformer or rejected as a duplicate, using a 1.00 Å RMSD cutoff to the heavy atoms in the central macrolide ring structure. Four low-energy conformers within 20 kJ/mol of the global minimum were found, and these were identical in water and octanol, though the ranking of conformers 3 and 4 was reversed. These four conformers were passed on to the docking program, in order to seed the calculation with a set of precomputed conformations to ensure that the conformational space available was thoroughly explored.

Next, the docking of the bryostatin 1 and C_9-Deoxy bryostatin 1 with the C1 domain of the PKC-δ was performed. The crystal structure of the C1b domain of PKC-δ was prepared for docking by adding hydrogen atoms and deleting the phorbol-13-acetate ligand. This was saved to a separate file to be used as a template for the similarity constraint (see below). Docking was done using the program GOLD 4.0 which uses a genetic algorithm to optimize the set of interactions between the ligand and the protein. Default settings were used for the genetic algorithm. The binding site was defined as a sphere with a 10.0 Å radius, centered on the Nε atom of residue Gln 257. For each ligand conformer, we performed 20 docking runs, with no early termination. We used the GoldScore scoring function with default parameters. All solutions with fitness score >

0.0 were kept. Free corners of ligand rings were allowed to flip above or below the plane of their neighboring atoms during docking, and intramolecular hydrogen bonds in the ligand were allowed to form. Torsion angle distributions were from the CSD. Additionally, we added a template similarity constraint with a weight of 30 to bias the conformation of docked ligands toward solutions where the acceptor atoms in the ligand were close in space to the acceptor atoms in phorbol. Finally the energy was minimized (Figure **1.74 and** Figure **1.75)**). The highest-scoring pose for each ligand was then further

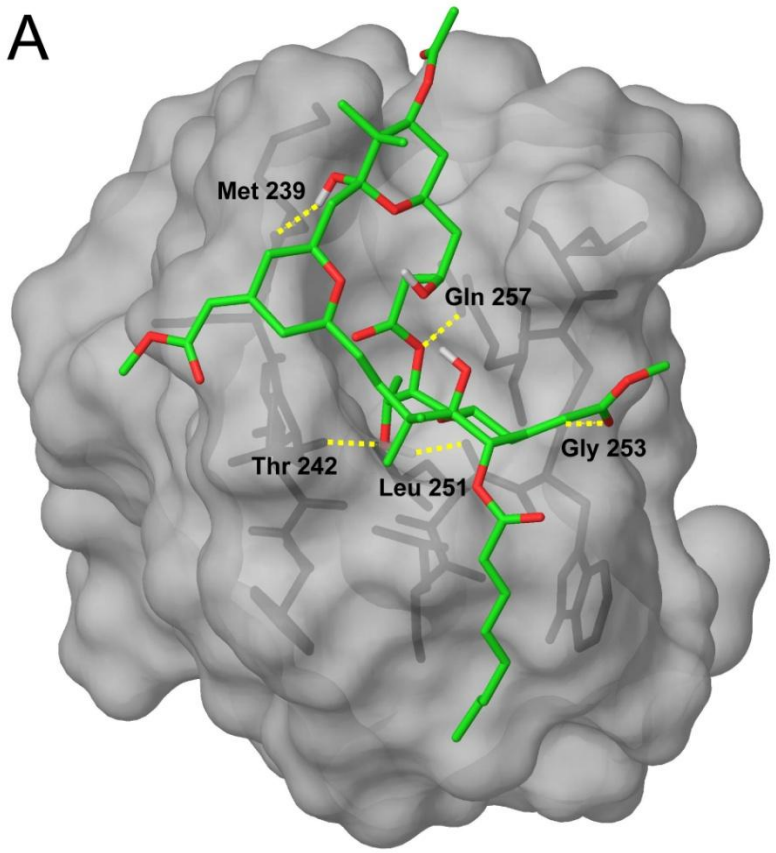


Figure 1.74. Structures of Bryostatin 1 Docked into the C1 Domain of PKCδ

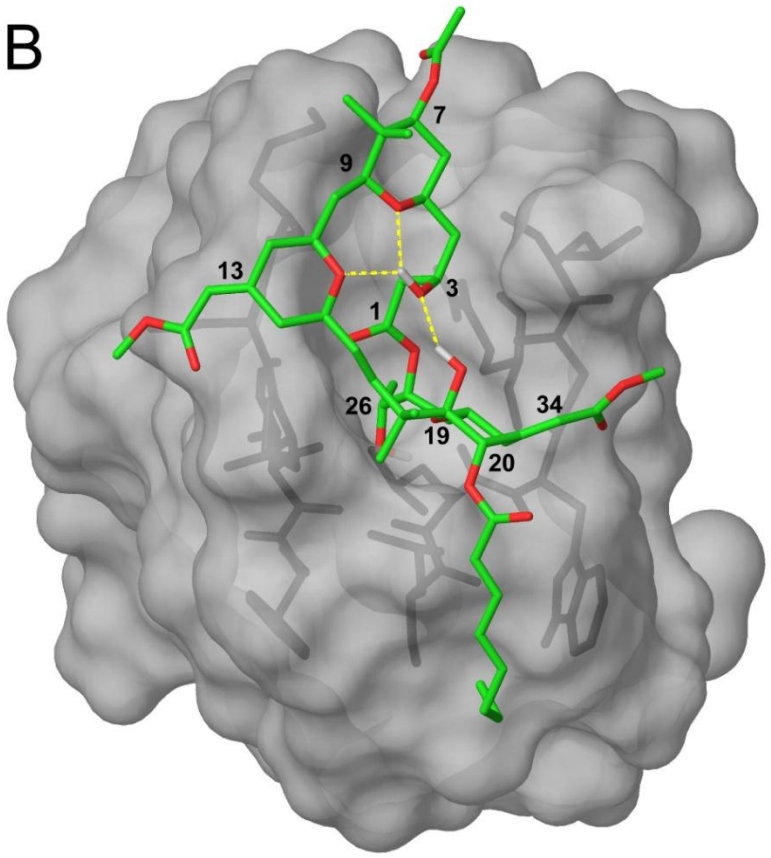
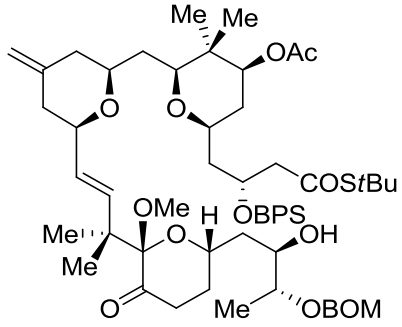


Figure 1.75. Structures of C_9-Deoxy Bryostatin Docked into the C1 Domain of PKCδ

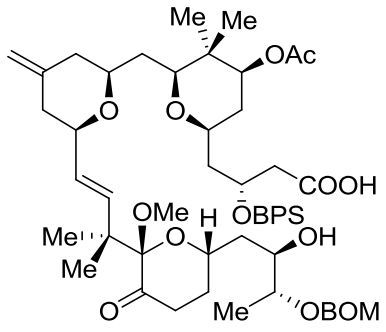
refined by energy minimization in MacroModel,[4] using the OPLS_2005 forcefield and octanol implicit solvent. All atoms in the C1 domain were held fixed, while ligand atoms were free to move, and the complex was minimized using the Polak-Ribiere conjugate gradient scheme to a gradient convergence of 0.05. Hydrogen bonds in the docked poses were preserved using distance constraints.The result of these docking studies revealed the similar conformation of both of the ligands.

Experimental Procedures and Analytical Data for Merle 32



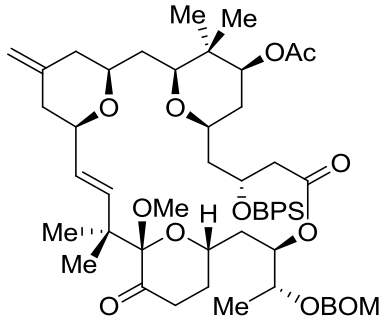
Preparation of (2*S*,4*S*,6*S*)-2-(((2*S*,6R)-6-((*E*)-3-((2*S*,6*S*)-6-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-hydroxybutyl)-2-methoxy-3-oxotetrahydro-2H-pyran-2-yl)-3-methylbut-1-enyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-6-((*R*)-2-(*tert*-butyldiphenylsilyloxy)-4-(*tert*-butylthio)-4-oxobutyl)-3,3-dimethyltetrahydro-2H-pyran-4-yl acetate (1.153): To a stirring solution of the PMB ether **1.131** (105 mg, 0.086 mmol, 1 equiv) in CH_2Cl_2 (7 mL) in a 25 mL flask at 0 ºC was added pH 7 phosphate buffer (1.6 mL). To the solution was added DDQ (98 mg, 0.43 mmol, 5 equiv) in one portion and the mixture was stirred vigorously. After stirring for 2 h at 0 ºC, the reaction was quenched by adding saturated aq. $NaHCO_3$ solution (5 mL) and stirring for 15 min. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated. Purification was accomplished with flash column chromatography, using a 1 x 10 cm silica gel column, eluting with 20% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (18 to 60) were combined and concentrated under reduced pressure to provide alcohol **1.153** (86 mg, 91%) as a white foam. R_f = 0.44 (50% EtOAc/hexanes); $[\alpha]_D^{20}$ = -22 (*c* = 1.5, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.66 (m, 4H), 7.45-7.30 (m, 11H), 5.99 (d, *J* = 16.0 Hz, 1H), 5.46 (dd, *J* = 16.1, 6.3 Hz, 1H), 4.90 (d, *J* = 7.3 Hz, 1H), 4.85 (d, *J* = 6.8 Hz, 1H), 4.71 (dd, *J*

= 10.0, 1.7 Hz, 1H), 4.68 (d, *J* = 11.7 Hz, 1H), 4.63 (d, *J* = 11.7 Hz, 1H), 4.45 (dd, *J* = 11.7, 4.8 Hz, 1H), 4.28-4.23 (m, 2H), 3.85 (ddd, *J* = 12.9, 6.3, 3.5 Hz, 1H), 3.80-3.76 (m, 1H), 3.62 (quin, *J* = 6.3 Hz, 1H), 3.45-3.41 (m, 1H), 3.33 (s, 3H), 3.04-3.02 (m, 1H), 2.86 (dd, *J* = 11.0, 1.7 Hz, 1H), 2.80 (d, *J* = 3.9 Hz, 1H), 2.64 (ddd, *J* = 14.6, 14.6, 6.8, Hz, 2H), 2.53 (quin, *J* = 9.2 Hz, 1H), 2.43 (dd, *J* = 6.8, 3.9 Hz, 1H), 2.39 (dd, *J* = 6.8, 3.4 Hz, 1H), 2.21 (s, 1H), 2.18 (s, 1H), 2.02 (s, 3H), 1.93-1.89 (m, 2H), 1.79-1.68 (m, 6H), 1.62 (ddd, *J* = 13.1, 10.7, 2.9 Hz, 1H), 1.57 (s, 2H), 1.43 (s, 9H), 1.31 (ddd, *J* = 12.9, 5.1, 3.1 Hz, 1H), 1.26 (d, *J* = 6.3 Hz, 3H), 1.25 (s, 3H), 1.09 (s, 3H), 1.02 (s, 9H), 0.79 (s, 3H), 0.72 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 207.2, 197.8, 170.7, 143.5, 137.5, 137.1, 136.0 (x2), 134.4, 133.8, 129.8, 129.7, 129.1, 128.6, 128.0 (x2), 127.8, 127.7, 109.5, 104.0, 94.0, 79.7, 79.1, 78.4, 77.0, 51.9, 48.0, 44.1, 43.6, 41.2, 39.6, 39.5, 37.5 (x2), 35.8, 33.8, 30.1, 30.0, 27.1, 22.9, 22.4, 22.2, 21.3, 19.6, 16.9, 13.6; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 51.9, 30.0, 22.9, 22.4, 22.1, 21.3, 16.9, 13.6; CH_2 δ 109.5, 94.0, 70.0, 52.7, 43.6, 41.2, 39.6, 39.5, 37.5, 35.8, 33.8, 30.1; CH_1 δ 137.1, 136.0 (x2), 129.8 (x2), 129.1, 128.6, 128.0 (x2), 127.8 (x2), 79.6, 79.1, 78.4, 77.0, 75.6, 73.0, 71.3, 70.0, 69.7, 69.4; CH_0 δ 207.2, 197.8, 170.7, 143.5, 137.5, 134.4, 133.8, 104.0, 48.0, 44.1, 39.5, 19.6; IR (neat) 2958, 2934, 2858, 2360, 2337, 1735, 1683, 1652, 1456, 1363, 1244, 1111, 1043, 740, 702 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{62}H_{90}NaO_{12}SNaSi$ (M+Na): 1121.5820, found: 1121.5817.



Preparation of (*R*)-4-((2*S*,4*S*,6*S*)-4-acetoxy-6-(((2*S*,6*R*)-6-((*E*)-3-((2*S*,6*S*)-6-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-hydroxy butyl)-2-methoxy-3-oxotetrahydro-2H-pyran-2-yl)-3-methylbut-1-enyl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-5,5-dimethyltetrahydro-2H-pyran-2-yl)-3-(*tert*-butyldiphenylsilyloxy)butanoic acid (1.154): To a stirring solution of thiolester **1.153** (86 mg, 0.078 mmol, 1.0 equiv) in a 4:1 mixture of THF/H_2O (4 mL, 0.02 M) at 0 ºC was added *m*CPBA (54 mg, 0.312 mmol, 4.0 equiv). The mixture was stirred at 0 ºC for 1 h and at rt for 2.5 h. The reaction was diluted with EtOAc (5 ml) and washed with saturated aq. $NaHSO_3$ solution (5 mL). The aqueous layer was extracted with EtOAc (3x5 mL). The combined organic layers were washed with brine (5 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to provide the crude seco acid. Purification was accomplished using flash column chromatography using 1 x 10 cm silica gel column using 30% EtOAc/hexanes collecting 4 mL fractions. The fractions from 6 to 16 provided starting material (22.6 mg, 26%) whereas those from 17 to 84 provided the desired seco acid **1.154** (51.6 mg, 64%) as a white foam. R_f = 0.44 (2:2:1 EtOAc/hexanes/MeOH); $[\alpha]_D^{20}$ = -5.8 (*c* = 1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.69-7.66 (m, 4H), 7.46-7.30 (m, 11H), 6.04 (d, *J* = 16.1 Hz, 1H), 5.44 (dd, *J* = 16.1, 6.3 Hz, 1H), 4.83 (dd, *J* = 15.1, 7.2 Hz, 2H), 4.70-4.66 (m, 2H), 4.63 (s, 2H), 4.45 (dd, *J* = 11.7, 4.8 Hz, 1H), 4.28-4.19 (m, 2H), 3.88 (ddd, *J* =

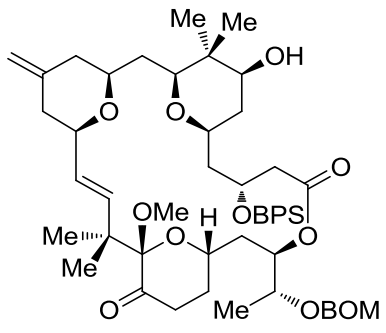
9.7, 6.8, 2.4 Hz, 1H), 3.77-3.72 (m, 1H), 3.66 (q, *J* = 5.8 Hz, 1H), 3.39-3.28 (m, 1H), 3.33 (s, 3H), 3.02-2.97 (m, 1H), 2.81 (dd, *J* = 11.5, 1.5 Hz, 1H), 2.70 (dd, *J* = 15.1, 4.8 Hz, 1H), 2.63-2.51 (m, 2H), 2.40 (ddd, *J* = 17.5, 5.8, 5.8 Hz, 1H), 2.17-2.14 (m, 2H), 2.03 (s, 3H), 2.02-1.99 (m, 1H), 1.94-1.89 (m, 2H), 1.81-1.62 (m, 7H), 1.54-1.50 (m, 2H), 1.40-1.36 (m, 2H), 1.25 (d, *J* = 5.8 Hz, 3H), 1.19 (s, 3H), 1.06 (s, 3H), 1.03 (s, 9H), 0.81 (s, 3H), 0.73 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 206.9, 174.2, 170.8, 143.8, 137.9, 137.5, 136.0 (x20, 133.9, 133.5, 130.0 (x2), 128.7, 128.6, 128.0 (x2), 127.9 (x2), 109.2, 103.6, 94.0, 80.2, 79.1, 78.4, 77.0, 76.1, 73.2, 71.2, 70.0, 69.7, 51.8, 43.9, 43.6, 41.2, 39.9, 39.2, 37.5, 37.3, 36.8, 33.9, 30.3, 27.1, 23.0, 22.3, 21.3, 19.4, 16.9, 13.5; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 51.9, 27.0, 23.0, 22.3, 21.3, 17.0, 13.5; CH_2 δ 109.2, 94.0, 70.0, 43.6, 43.0, 41.2, 39.9, 39.2, 37.3, 36.1, 33.9, 30.3; CH_1 δ 137.9, 136.0 (x2), 130 (x2), 128.7, 128.6, 128.0 (x2), 127.9 (x2), 80.2, 79.7, 77.0, 76.1, 73.2, 71.1, 69.7, 69.1; CH_0 δ 206.9, 174.2, 170.8, 143.8, 137.9, 103.6, 79.1, 43.9, 36.8, 19.4; IR (neat) 2934, 2892, 2858, 2337, 1733, 1718, 1427, 1384, 1244, 1111, 1027, 753, 703 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{59}H_{82}O_{13}NaSi$ (M+Na): 1049.5422, found: 1049.5415.



Preparation of (1*S*,3*S*,7*R*,11*S*,15*S*,17*R*, 21*R*,23*S*,25*S*,*E*)-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-21-((*tert*-butyldiphenylsilyl) oxy)-11-methoxy-10,10,26,26-tetramethyl-5-methylene-12,19-dioxo-18,27,28,29-tetraoxatetracyclo [21.3.1.13,7.111,15] nonacos-8-en-25-yl acetate (1.155): To a stirring solution of the seco acid **1.154** (63.6 mg, 0.061 mmol, 1.0 equiv) in THF (2 mL)

in a 5 mL vial at 0 °C was added triethylamine (48 µL, 0.37 mmol, 6.0 equiv) and 2,4,6-trichlorobenzoyl chloride (29 µL, 0.185 mmol, 3.0 equiv). After 1 h, the reaction was warmed to rt and stirring continued for additional 5 h. The reaction mixture was diluted with 3:1 toluene/ THF (20 mL) and taken up in a 25 mL gas-tight syringe. This solution was added by syringe pump to a stirring solution of DMAP (151mg, 1.23 mmol, 20.0 equiv) in toluene (41 mL) at 45 °C over 12 h. The residual contents of the syringe were rinsed into the flask with toluene (2 mL) and stirring continued for an additional 2 h. The reaction mixture was cooled to rt and washed with saturated aqueous $NaHCO_3$ solution (2 x 10 mL) and brine (2 x 10 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 1 x 11 cm silica gel column, eluting with 15% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (25-41) were combined and concentrated under reduced pressure to provide pure macrolactone **1.155** as a white foam (50 mg, 81%): R_f = 0.37 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = -1.3 (*c* = 1.1, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.71-7.69 (m, 4H), 7.46-7.28 (m, 11H), 6.24 (d, *J* = 15.6 Hz, 1H), 5.46 (ddd, *J* = 11.8, 3.4, 2.6 Hz, 1H), 5.39 (dd, *J* = 16.1, 8.3 Hz, 1H), 4.89 (d, *J* = 6.8 Hz, 1H), 4.85-4.84 (m, 2H), 4.81 (d, *J* = 6.8 Hz, 1H), 4.66 (s, 2H), 4.65 (ddd, *J* = 11.5, 7.3, 6.5 Hz, 1H), 4.25 (dd, *J* = 11.2, 4.8 Hz, 1H), 4.21 (dd, *J* = 6.3, 3.9 Hz, 1H), 4.03 (ddd, *J* = 14.6, 7.8, 3.9 Hz, 1H), 3.92-3.87 (m, 1H), 3.29-3.27 (m, 1H), 3.24 (s, 3H), 2.66 (ddd, *J* = 14.6, 9.7, 5.3 Hz, 1H), 2.55 (dd, *J* = 14.6, 8.3 Hz, 1H), 2.50 (dd, *J* = 15.1, 3.9 Hz, 1H), 2.43 (d, *J* = 9.2 Hz, 1H), 2.39-2.33 (m, 2H), 2.24 (t, *J* = 14.6 Hz, 2H), 2.13-2.03 (m, 3H), 1.99 (s, 3H), 1.91-1.82 (m, 3H), 1.50-1.37 (m, 4H), 1.32 (s, 3H), 1.18 (d, *J* = 6.3 Hz, 3H), 1.09 (ddd, *J* = 11.7, 4.3, 1.9 Hz, 1H), 1.00 (s, 3H), 0.99 (s, 9H), 0.72 (s,

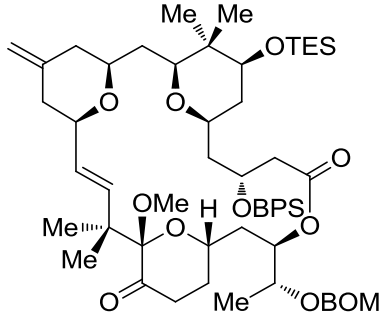
3H), 0.68 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 205.7, 171.4, 170.6, 137.9, 136.1, 135.8, 135.2, 134.6, 133.4, 130.0, 129.8, 128.5, 128.3, 128.0, 127.9, 127.8, 127.4, 109.2, 102.2, 93.6, 81.1, 80.6, 77.0, 76.0, 73.9, 71.9, 70.8, 69.7, 65.7, 52.6, 44.8, 43.5 (x2), 42.8, 41.3, 40.6, 37.9, 36.0, 33.4, 31.0, 26.9, 24.6, 22.3, 21.2, 19.8, 19.4, 14.7, 13.9, 12.5; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 52.6, 26.9, 24.6, 22.2, 21.2, 19.8, 14.7, 13.9, 12.5; CH_2 δ 109.2, 93.6, 69.6, 44.8, 43.5, 40.6, 37.8, 36.0, 33.4, 33.2, 31.0; CH_1 δ 141.1, 136.1, 135.8, 130.0, 129.8, 128.9, 128.5, 128.3, 128.0, 127.7, 127.4, 81.1, 80.6, 76.9, 76.0, 73.9, 71.9, 70.8, 69.7; CH_0 δ 205.7, 171.4, 170.6, 137.9, 135.2, 134.6, 102.2, 65.7, 42.8, 37.3, 19.4; IR (neat) 3070, 2970, 2734, 2888, 2360, 2337, 1734, 1651, 1576, 1419, 1363, 1244, 1109, 1039, 879, 819, 738, 702, 667 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{59}H_{80}NaO_{12}Si$ (M+Na): 1031.5317, found: 1031.5313.



Preparation of (1*S*,3*S*,7*R*,11*S*,15*S*,17*R*,21*R*,23*S*,25*S*,*E*)-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-21-((*tert*-butyldiphenylsilyl)oxy)-25-hydroxy-11-methoxy-10,10,26,26-tetramethyl-5-methylene-18,27,28,29-tetraoxatetracyclo[21.3.1.13,7.111,15] nonacos-8-ene-12,19-dione (1.156): To a stirring solution of acetate **1.155** (20 mg, 0.019 mmol, 1.0 equiv) in 1 mL of MeOH in a 4 mL vial at room temperature was added K_2CO_3 (13.6 mg, 0.099 mmol, 5 equiv) and the mixture was stirred for 6 h. The reaction was quenched by addition of 5 mL of saturated aqueous NH_4Cl solution and was then diluted with 5 mL of 40% EtOAc/hexanes. The phases were separated and the aqueous phase was extracted with 40% EtOAc/hexanes (2

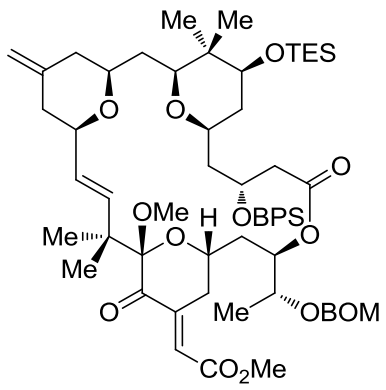
x 5 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 1 x 9 cm silica gel column, eluting with 15% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (11-42) were combined and concentrated under reduced pressure to provide alcohol **1.156** (18 mg, 94%) as white foam. R_f = 0.4 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = -2 (*c* = 1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.72-7.70 (m, 4H), 7.45-7.27 (m, 11H), 6.23 (d, *J* = 15.6 Hz, 1H), 5.46 (ddd, *J* = 11.2, 3.9, 1.9 Hz, 1H), 5.40 (dd, *J* = 15.6, 8.3 Hz, 1H), 4.89 (d, *J* = 6.8 Hz, 1H), 4.84 (dd, *J* = 7.8, 1.4 Hz, 2H), 4.82 (d, *J* = 6.8 Hz, 1H), 4.66 (s, 2H), 4.50 (ddd, *J* = 9.6, 9.6, 3.9 Hz, 1H), 4.24-4.19 (m, 1H), 4.04 (ddd, *J* = 15.1, 8.3, 4.3 Hz, 1H), 3.89 (ddd, *J* = 14.4, 7.0, 1.9 Hz, 1H), 3.28-3.26 (m, 1H), 3.25 (s, 3H), 2.97 (ddd, *J* = 10.7, 5.3, 5.3 Hz, 1H), 2.66 (ddd, *J* = 14.6, 9.2, 5.3 Hz, 1H), 2.59-2.50 (m, 2H), 2.39-2.32 (m, 3H), 2.28-2.21 (m, 2H), 2.14-1.98 (m, 3H), 1.92-1.83 (m, 3H), 1.51-1.37 (m, 4H), 1.32 (s, 3H), 1.18 (d, *J* = 6.3 Hz, 3H), 1.17-1.15 (m, 1H), 1.01 (s, 3H), 0.99 (s, 9H), 0.78 (s, 3H), 0.63 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 205.8, 171.5, 144.3, 141.0, 138.0, 136.2, 135.9, 134.8, 133.7, 129.9, 129.7, 128.5, 128.1, 127.9, 127.7, 127.5, 109.1, 93.6, 81.1, 80.6, 76.2, 75.6, 74.1, 72.0, 70.9, 70.8, 69.7, 65.8, 52.6, 44.9, 43.7, 43.5, 41.1, 40.7, 38.5, 38.0, 36.9, 36.1, 33.2, 31.0, 27.0, 24.6, 22.3, 19.9, 19.5, 14.8, 12.3; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 52.6, 27.0, 24.6, 22.3, 19.9, 14.8, 12.4; CH_2 δ 109.1, 93.6, 69.7, 44.9, 43.7, 41.4, 40.7, 38.0, 36.9, 36.1, 33.2, 31.0; CH_1 δ 141.0, 136.1, 135.9, 129.8, 129.7, 128.5, 128.1, 127.9, 127.8, 127.7, 127.5, 81.1, 80.6, 76.2, 75.6, 74.1, 72.0, 70.9, 70.8; CH_0 δ 205.8, 171.5, 144.3, 138.0, 134.8, 133.7, 65.8, 43.5, 38.5, 19.5; IR (neat) 3069, 2934, 2889, 2858, 1735, 1651, 1471, 1427, 1276, 1312, 1239, 1165, 1109, 1042, 739, 703

cm^{-1}; HRMS (ESI/APCI) calcd for $C_{59}H_{80}NaO_{12}Si$ (M+Na): 1031.5317, found: 1031.5313.



Preparation of (1*S*,3*S*,7*R*,11*S*,15*S*, 17*R*,21*R*,23*R*,25*S*,*E*)-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-21-((*tert*-butyldiphenyl silyl)oxy)-11-methoxy-10,10,26,26-tetramethyl-5-methylene-25-((triethylsilyl)oxy)-18,27,28,29-tetraoxatetracyclo [21.3.1.13,7.111,15] nonacos-8-ene-12,19-dione (1.157): To a stirring solution of alcohol **1.156** (13 mg, 0.013 mmol, 1.0 equiv) in 670 µL of CH_2Cl_2 in a 4 mL vial at 0 °C was added Et_3N (10 µL , 0.08 mmol, 6 equiv) followed by a small crystal of DMAP. To the reaction mixture was then added TESCl (6 µL, 0.04 mmol, 3 equiv) and stirred at 0 °C for 1 h. The reaction was warmed to rt and stirred overnight. The reaction was quenched by addition of 5 mL of water and was then diluted with 5 mL of CH_2Cl_2. The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 1 x 7 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (2-8) were combined and concentrated under reduced pressure to provide TES ether **1.157** (13.5 mg, 93%) as colorless oil. R_f = 0.63 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = -4.7 (*c* = 1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.72-7.69 (m, 4H), 7.45-7.29 (m, 11H), 6.22 (d, *J* = 15.6

Hz, 1H), 5.47 (ddd, *J* = 10.4, 4.0, 2.1 Hz, 1H), 5.39 (dd, *J* = 16.1, 8.3 Hz, 1H), 4.90 (d, *J* = 6.8 Hz, 1H), 4.82 (d, *J* = 6.3 Hz, 2H), 4.84-4.82 (m, 2H), 4.66 (s, 2H), 4.54-4.49 (m, 1H), 4.25-4.21 (m, 1H), 4.05 (ddd, *J* = 14.6, 7.8, 4.3 Hz, 1H), 3.88 (ddd, *J* = 11.2, 8.4, 2.0 Hz, 1H), 3.25 (s, 3H), 2.95 (ddd, *J* = 11.2, 4.3 Hz, 1H), 2.66 (ddd, *J* = 14.6, 9.2, 5.3 Hz, 1H), 2.56-2.54 (m, 2H), 2.39-2.18 (m, 5H), 2.12 (dd, *J* = 13.6, 7.8 Hz, 1H),, 2.08-1.98 (m, 2H), 1.92-1.81 (m, 3H), 1.49-1.34 (m, 3H), 1.31 (s, 3H), 1.27-1.22 (m, 2H), 1.18 (d, *J* = 6.3 Hz, 3H), 1.01 (s, 3H), 1.00 (s, 9H), 0.91 (t, *J* = 7.8 Hz, 9H), 0.82 0.78 (m, 1H), 0.70 (s, 3H), 0.61 (s, 3H), 0.50 (q, *J* = 7.8 Hz, 6H) ; 125 MHz ^{13}C NMR ($CDCl_3$) δ 205.8, 171.6, 144.4, 141.0, 138.0, 135.9, 135.0, 133.9, 129.8, 128.5, 128.1, 127.8, 127.7, 127.5, 109.0, 102.5, 93.6, 81.2, 80.6, 76.0, 74.0, 71.9, 71.2, 70.7, 69.7, 65.7, 52.6, 45.0, 43.8, 43.5, 41.4, 40.7, 38.8, 38.2, 37.5, 36.0, 33.1, 31.1, 27.0, 24.6, 22.7, 19.8, 19.5, 14.7, 12.6, 7.1, 5.2; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 52.6, 27.0, 24.6, 22.7, 19.8, 14.7, 12.6, 7.1; CH_2 δ 109.0, 93.6, 69.7, 45.0, 43.8, 41.4, 40.7, 38.2, 37.5, 36.5, 36.0, 33.1, 31.0, 5.2; CH_1 δ 141.0, 136.1, 135.9, 129.8, 129.7, 128.5, 128.1, 127.8, 127.7, 127.5, 81.2, 80.6, 76.2, 76.0, 74.0, 71.9, 71.2, 70.7; CH_0 δ 205.8, 171.6, 144.4, 138.0, 135.0, 133.9, 102.5, 65.7, 38.8, 19.5; IR (neat) 2955, 2876, 1735, 1460, 1427, 1362, 1311, 1236, 1165, 1105, 1036, 839, 737, 702 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{63}H_{92}NaO_{11}Si2$ (M+Na): 1103.6076, found: 1103.6093.



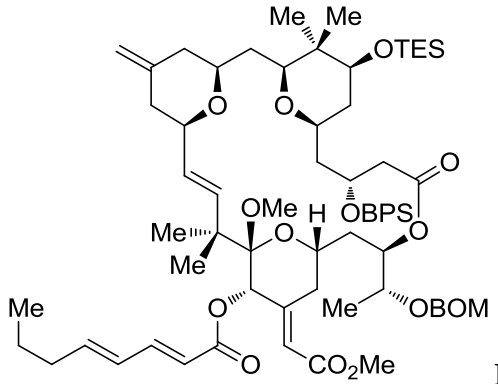
Preparation of (*E*)-methyl 2-((1*S*,3*S*,7*R*,11*S*, 15*S*,17*R*,21*R*,23R,25*S*,*E*)-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-21-((*tert*-butyl diphenylsilyl)oxy)-11-methoxy-10,10,26,26-tetramethyl-5-methylene-12,19-dioxo-25-((triethylsilyl)oxy)-18,27,28,29-tetraoxatetracyclo [21.3.1.13,7.111,15] nonacos-8-en-13-ylidene)acetate (1.159): To a stirring solution of (iPr)$_2$NH (0.27 mL, 1.93 mmol) in 6 mL of THF in a 25 mL rb flask at -78 ºC was added *n*-BuLi (2.61 M in hexanes, 0.67 mL, 1.75 mmol) via syringe. The solution stirred at -78 ºC for 30 min and was then allowed to warm to 0 ºC for 20 min. This 0.25 M LDA solution was used immediately in the following aldol reaction.

To a stirring solution of ketone **1.157** (9 mg, 0.083 mmol, 1.0 equiv) in THF (277 µL, 0.03 M) in a 4 mL vial at -78 ºC was added a 0.25 M solution of LDA in THF (66 µL, 0.016 mmol, 2 equiv) slowly via syringe down the side of the vial. The resulting light-yellow reaction mixture was allowed to stir at -78 ºC for 10 min and a freshly prepared solution of methyl glyoxylate (ca 3.0 M in THF, 55 µL, 1.66 mmol, 20.0 equiv) was added slowly via syringe down the side of the flask upon which the yellow color of the solution disappeared. The reaction mixture stirred at -78 ºC for 40 min and was quenched by addition of 2 mL of saturated aqueous NH_4Cl solution. The mixture was allowed to warm to rt and was then partitioned between 5 mL of EtOAc and 5 mL of brine. The phases were separated and the aqueous phase was extracted with EtOAc (3 x

5 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 1 x 5 cm silica gel column, eluting with 10% EtOAc/hexanes (50 mL) then 30% EtOAc/hexanes (50 mL), collecting 4 mL fractions. Fractions 4-12 gave unreacted starting material which were combined and concentrated to provide 1.5 mg of the starting ketone **1.157.** The product containing fractions (13-24) were combined and concentrated under reduced pressure to provide the intermediate aldol adduct as a mixture of diastereomers (7.6 mg, 78%). This material was taken into the following elimination reaction.

To a stirring solution of the aforementioned aldol adduct (5 mg, 0.0042 mmol, 1.0 equiv) in pyridine (400 µL) in a 5 mL reaction vial at rt was added a 0.1 M solution of DMAP in CH_2Cl_2 (42 µL, 0.0047 mmol, 1.0 equiv), and 0.5 M solution of Ac_2O in CH_2Cl_2 (170 µL, 0.085 mmol, 1.0 equiv),. The reaction mixture was allowed to stir at rt for 24 h and was then quenched by addition of saturated aqueous $NaHCO_3$ solution (2 mL). The mixture was diluted with 5 mL CH_2Cl_2 and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (2 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 1 x 5 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 4 ml fractions. The product containing fractions (6-12) were combined and concentrated under reduced pressure to provide pure enoate **1.159** (4.6 mg, 93%) as a clear light-yellow oil: R_f = 0.62 (40% EtOAc/hexanes); $[\alpha]_D^{20}$ = -17 (*c* = 0.4, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.78-7.68 (m, 4H), 7.44-7.25 (m, 11H), 6.54 (s, 1H), 5.97 (d, *J* = 16.1 Hz, 1H), 5.36 (dd, *J* = 15.6, 6.8 Hz, 1H), 5.30

(ddd, *J* = 9.3, 3.4, 3.4 Hz, 1H), 4.85 (d, *J* = 7.3 Hz, 1H), 4.82 (d, *J* = 6.8 Hz, 1H), 4.81-4.77 (m, 2H), 4.65 (s, 2H), 4.49-4.47 (m, 1H), 4.12-4.09 (m, 2H), 4.03 (dd, *J* = 6.3, 3.9 Hz, 1H), 3.72 (s, 3H), 3.68-3.67 (m, 1H), 3.46-3.3.42 (m, 1H), 3.26 (s, 3H), 3.03 (dd, *J* = 10.7, 2.9 Hz, 1H), 2.99 (dd, *J* = 10.7, 2.9 Hz, 1H), 2.78 (dd, *J* = 11.2, 4.8 Hz, 1H), 2.73 (dd, *J* = 11.2, 4.8 Hz, 1H), 2.69-2.67 (m, 1H), 2.62 (dd, *J* = 15.6, 3.9 Hz, 1H), 2.29-2.25 (m, 1H), 2.21-2.08 (m, 2H), 2.03 (ddd, *J* = 14.6, 9.7, 4.8 Hz, 1H), 1.98-1.83 (m, 2H), 1.58-1.34 (m, 7H), 1.24 (d, *J* = 7.3 Hz, 3H), 1.23 (s, 3H), 0.97 (s, 12H), 0.90 (t, *J* = 8.3, 3H), 0.67 (s, 3H), 0.63 (s, 3H), 0.47 (q, *J* = 8.2 Hz, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 195.9, 171.3, 166.1, 148.2, 144.5, 137.9, 137.7, 1366.2, 136.0, 134.8, 133.8, 129.8, 129.7, 129.3, 128.5, 128.0, 127.9, 127.8, 127.7, 123.0, 108.9, 103.9, 93.6, 81.3, 79.5, 76.4, 76.1, 73.2, 73.0, 71.2, 69.8, 69.7, 69.0, 52.5, 51.9, 44.4, 43.9, 43.7, 41.5, 40.9, 39.0, 37.7, 37.4, 34.2, 33.9, 27.0, 23.4, 22.8, 21.1, 19.6, 15.3, 12.7, 7.1, 5.2; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ;52.5, 51.9, 27.0, 23.4, 22.8, 21.2, 15.3, 12.7, 7.; CH_2 δ 108.9, 93.7, 69.7, 44.0, 43.7, 41.5, 40.9, 37.7, 37.4, 34.2, 33.9, 5.3; CH_1 δ 137.7, 136.2, 136.0, 129.8, 129.7, 129.4, 128.6, 128.0, 127.9, 127.8, 127.7, 123.1, 81.3, 79.5, 76.5, 76.1, 73.2, 73.0, 71.2, 69.8, ; CH_0 δ 195.9, 171.3, 166.1, 148.2, 144.5, 137.9, 134.8, 129.7, 103.9, 69.0, 43.9, 19.6; IR (neat) 2970, 2936, 1734, 1513, 1455, 1378, 1248, 1170, 1103, 1036, 824, 741, 700 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{66}H_{94}NaO_{13}Si2$ (M+Na): 1173.6131, found: 1173.6129.

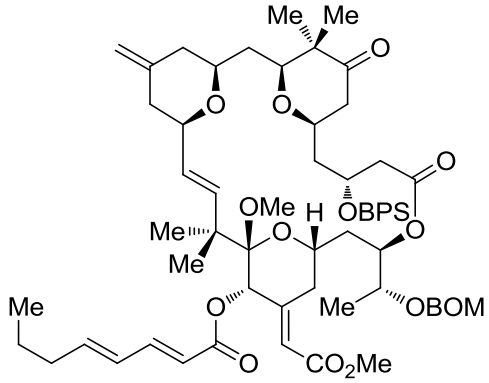


Preparation of (2*E*,4*E*)-(1*S*,3*S*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*R*,25*S*)-17-((*R*)-1-((benzyloxy) methoxy)ethyl)-21-((*tert*-butyldiphenylsilyl)oxy)-11-methoxy-13-(2-methoxy-2-oxoethylidene)-10,10,26,26-tetramethyl-5-methylene-19-oxo-25-((triethylsilyl)oxy)-18,27,28,29-tetra oxatetracyclo [21.3.1.13,7.111,15]nonacos-8-en-12-yl octa-2,4-dienoate (1.160): To a stirring solution of ketone **1.159** (9.5 mg, 0.008 mmol, 1.0 equiv) in MeOH (800 µL, 0.01 M) in a 5 mL reaction vial at rt was added $CeCl_3 \cdot 7H_2O$ (61 mg, 0.16 mmol, 20.0 equiv). The mixture was stirred until all the $CeCl_3 \cdot 7H_2O$ was completely dissolved. The mixture was then cooled to -42 ºC and stirred for 10 min and then $NaBH_4$ (3 mg, 0.08 mmol, 10.0 equiv) was added. Stirring continued for 1 h at -42 ºC after which the reaction was quenched by slow addition of saturated aqueous NH_4Cl solution (2 mL) and diluted with 40% EtOAc/hexanes (5 mL). The layers were separated and the aqueous layer was extracted with 40% EtOAc/hexanes (3 x 5 mL). The organic phase was washed with brine (5 mL), then dried over Na_2SO_4, filtered and concentrated under reduced pressure to provide the crude intermediate alcohol which was carried directly to the next step without purification.

To a stirring solution of this alcohol in CH_2Cl_2 (800 µL, 0.001 M) in a 5 mL reaction vial at rt was added pyridine (33 µL, 0.41 mmol, 50 equiv), DMAP (10 mg, 0.08 mmol, 10 equiv), and octadienoic anhydride (66 mg, 0.24 mmol, 30 equiv). The reaction

mixture stirred at rt for 12 h and was then quenched by the addition of saturated aqueous $NaHCO_3$ solution (2.0 mL). The mixture was stirred vigorously for 30 min and was then diluted with 40% EtOAc/hexanes (5mL). The phases were separated and the aqueous phase was extracted with 40% EtOAc/hexanes (3 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography using 10% EtOAc/hexanes (50 mL) followed by 20% EtOAc/hexanes collecting 4 mL fractions. Fractions 16-23 provided the desired product as 4.2:1 mixture of diastereomers which were further separated by a preparative TLC with 4% acetone/benzene to provide the ester **1.160** (8.6 mg, 82%, 2 steps) as a pale yellow liquid. R_f = 0.46 (30% EtOAc/hexanes); $[\alpha]_D^{20} = +2.5$ (*c* = 0.5, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.72-7.70 (m, 4H), 7.45-7.24 (m, 11H), 6.19-6.14(m, 2H), 5.98 (d, *J* = 1.4 Hz, 1H), 5.77 (d, *J* = 15.1 Hz, 1H), 5.64-5.61 (m, 1H), 5.29 (dd, *J* = 15.1, 8.7 Hz, 1H), 5.23 (s, 1H), 4.94 (d, *J* = 6.8 Hz, 1H), 4.86-4.84 (m, 2H), 4.73 (d, *J* = 11.7 Hz, 1H), 4.69 (d, *J* = 11.7 Hz, 1H), 4.52-4.51 (m, 1H), 4.26-4.19 (m, 1H), 3.85 (t, *J* = 10.7 Hz, 1H), 3.75-3.72 (m, 1H), 3.69 (s, 3H), 3.19 (t, *J* = 8.7, 1H), 3.08 (s, 3H), 2.92 (dd, *J* = 11.2, 4.3 Hz, 1H), 2.51 (dd, *J* = 15.1, 9.7 Hz, 1H), 2.42 (dd, *J* = 15.1, 1.9 Hz, 1H), 2.29-2.13 (m, 7H), 2.05 (t, *J* = 13.1 Hz, 1H), 2.00 (t, *J* = 6.8 Hz, 1H), 1.82 (t, *J* = 12.2 Hz, 1H), 1.49-1.26 (m, 5H), 1.19 (d, *J* = 6.3, 1H), 1.10 (s, 3H), 1.06 (s, 3H), 1.00 (s, 9H), 0.95-0.93 (m, 3H), 0.91 (t, *J* = 7.8, 9H), 0.76-0.73 (m, 1H), 0.69 (s, 3H), 0.58 (s, 3H), 0.49 (q, *J* = 8.3 Hz, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 171.8, 167.0, 165.5, 151.7, 146.6, 145.8, 144.5, 142.4, 138.0, 136.2, 135.9, 135.1, 134.0, 131.0, 129.8, 129.6, 128.9, 128.1, 127.8, 127.7, 125.4, 119.4, 118.6, 109.0, 103.4, 93.6, 81.3, 81.2, 76.5, 75.9, 74.0, 73.3, 72.1, 70.0, 69.7, 67.2, 53.0, 52.3, 45.3 (x2), 44.0, 41.4, 40.6, 38.8,

38.2, 37.6, 35.2, 33.4, 31.1, 29.9, 27.0, 25.8, 22.7, 22.0, 19.5, 14.7, 13.9, 12.5, 7.1, 5.3; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 53.0, 51.3, 27.0, 25.8, 22.7, 14.7, 13.9, 12.5, 7.1; CH_2 δ 109.0, 93.6, 69.7, 45.3, 44.0, 41.4, 40.6, 38.2, 37.6, 35.2, 33.4, 31.1, 22.0, 5.3; CH_1 δ 146.6, 145.8, 142.4, 136.2, 135.9, 129.8, 129.6, 128.5, 128.1, 127.8, 127.7, 125.5, 119.4, 118.7, 81.2, 76.5, 75.9, 74.0, 73.7, 72.1, 71.2, 70.0, 67.2; CH_0 δ; 171.8, 167.0, 165.5, 151.7, 144.5, 138.0, 131.0, 128.9, 103.4, 38.8, 29.9, 19.5; IR (neat) 2933, 1760, 1470, 1427, 1378, 1246, 1111, 1038, 843, 740, 702, 677 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{74}H_{106}NaO_{14}Si_2$(M+Na): 1297.7019, found: 1297.7025.

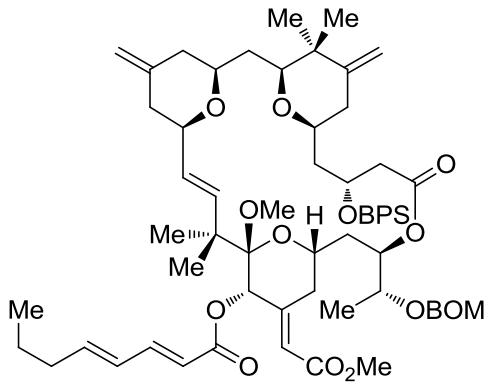


Preparation of (2*E*,4*E*)-(1*S*,3*S*,7*R*,8*E*, 11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*R*)-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-21-((*tert*-butyldiphenylsilyl)oxy)-11-methoxy-13-(2-methoxy-2-oxoethylidene)-10,10,26,26-tetramethyl-5-methylene-19,25-dioxo-18,27,28,29-tetraoxatetracyclo [21.3.1.13,7.111,15]nonacos-8-en-12-yl octa-2,4-dienoate (1.161): To a stirring solution of ketone TES ether **1.160** (6.3 mg, 0.0049 mmol, 1.0 equiv) in MeOH (250 µL, 0.02 M) in a 5 mL reaction vial at rt was added 0.1 M solution of PPTS in MeOH (25 µL, 0.0024 mmol, 0.5 equiv). The mixture was stirred at rt for 48 h after which the reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution (2 mL) and diluted with EtOAc (5mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 5mL). The organic phase was washed with brine (5 mL), then dried over

Na_2SO_4, filtered and concentrated under reduced pressure to provide crude intermediate alcohol which was carried directly to the next step without purification.

To a stirring solution of the aftermentioned intermediate alcohol in CH_2Cl_2 (200 µL, 0.01 M) in a 5 mL reaction vial at 0 ºC was added pyridine (3 µL, 0.014 mmol, 20 equiv) followed by Dess-Martin reagent (1.7 mg, 0.0041 mmol, 2 equiv). The reaction mixture was stirred at 0 ºC for 45 min and at rt for 15 min. The reaction was then quenched by the addition of saturated aqueous $NaHCO_3$ solution (5.0 mL) and diluted with 40% EtOAc/hexanes (5mL). The phases were separated and the aqueous phase was extracted with 40% EtOAc/hexanes (3 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography using 15% EtOAc/hexanes collecting 2 mL fractions. The fractions from 17-24 provided the desired product. One more batch of the same was reaction was repeated with same amount of starting material to provide ketone **1.161** (4.8 mg, 84%, 2 steps) as a pale yellow liquid. R_f = 0.58 (30% EtOAc/hexanes); Analytial data: $[\alpha]_D^{20} = +13.6$ (*c* = 0.2, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.69 (m, 4H), 7.44-7.28 (m, 11H), 6.21-6.16 (m, 2H), 5.99 (s, 1H), 5.78 (d, *J* = 16.5 Hz, 1H), 5.64-5.63 (m, 1H), 5.30 (dd, *J* = 15.6, 8.2 Hz, 1H), 5.24 (s, 1H), 4.92 (d, *J* = 6.8 Hz, 1H), 4.87 (s, 2H), 4.84 (d, *J* = 6.8 Hz, 1H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.68 (d, *J* = 12.1 Hz, 1H), 4.55-4.50 (m, 1H), 4.19-4.17 (m, 1H), 4.07 (d, *J* = 4.3 Hz, 1H), 3.88 (t, *J* = 10.2 Hz, 1H), 3.75-3.73 (m, 1H), 3.69 (s, 3H), 3.23 (t, *J* = 7.8, 1H), 3.11 (s, 3H), 2.61-2.54 (m, 2H), 2.41 (dd, *J* = 14.4, 1.2 Hz, 1H), 2.24 (t, *J* = 11.7 Hz, 1H), 2.20-2.15 (m, 2H), 2.08-1.99 (m, 2H), 1.85 (t, *J* = 11.7 Hz, 1H), 1.59-1.40 (m, 4H), 1.32-1.31 (m, 4H), 1.26 (s, 3H), 1.19 (d, *J* = 6.4, 1H), 1.12 (s, 3H), 1.08 (s, 3H), 0.98 (s,

9H), 0.92 (t, J = 7.3, 3H), 0.90 (s, 3H), 0.58 (s, 3H), 0.84 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 211.0, 171.4, 167.0, 165.6, 151.7, 146.7, 145.9, 144.2, 142.4, 138.1, 136.2, 135.9, 134.6, 133.5, 130.0, 129.9, 128.6, 128.1, 128.0, 127.9, 127.8, 125.5, 119.5, 118.7, 109.3, 103.4, 93.7, 81.7, 81.3, 76.1, 75.4, 73.7, 73.6, 72.3, 70.2, 69.7, 67.3, 52.9, 51.3(x2), 48.9, 45.3, 45.1, 44.3, 41.4, 40.6, 38.3, 35.2, 33.7, 31.1, 29.9, 27.0, 25.8, 22.0, 20.3, 19.5, 19.1, 18.7, 14.8, 13.8; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 52.9, 51.3, 27.0, 25.8, 20.2, 19.1, 18.6, 14.8, 13.8; CH_2 δ 109.3, 93.7, 69.7, 45.1, 44.3, 41.4, 40.6, 38.3, 35.2, 33.7, 31.1, 29.9, 22.0; CH_1 δ 146.7, 145.9, 142.3, 136.2, 135.9, 130.0, 129.9, 128.6, 128.5, 128.1, 128.0, 127.9, 127.8, 125.5, 119.4, 118.7, 81.7, 81.3, 76.1, 75.4, 73.7, 72.2, 70.2, 63.7; CH_0 δ; 211.0, 171.4, 167.0, 165.6, 151.7, 144.2, 138.1, 134.6, 103.4, 73.6, 48.9, 45.3, 19.5; IR (neat) 2950, 1715, 1609, 1513, 1454, 1382, 1249, 1171, 1102, 1038, 846, 739, 698 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{68}H_{90}NaO_{14}Si$(M+Na): 1181.5998, found: 1181.6001.

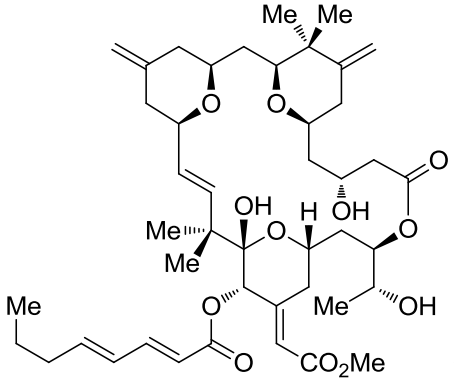


Preparation of (2*E*,4*E*)-(1*S*,3*S*,7*R*,8*E*, 11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*S*)-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-21-((tert-butyldiphenylsilyl)oxy)-11-methoxy-13-(2-methoxy-2-oxoethylidene)-10,10,26,26-tetramethyl-5,25-dimethylene-19-oxo-18,27,28,29-tetraoxatetracyclo [21.3.1.13,7.111,15]nonacos-8-en-12-yl octa-2,4-dienoate (1.162): To a stirring solution of methyltriphenylphosphonium bromide (recrystallized from hexanes/CH_2Cl_2 and dried

under high vacuum at 100 ºC overnight; 180 mg, 0.5 mmol, 1.0 equiv) in 5 mL THF at rt was added a 2.5 M solution of *n*-BuLi in THF (181 mL, 0.45 mmol, 0.9 equiv) dropwise via a syringe during which the white salt was barely left in the solution and the color of solution changed to yellow. The reaction mixture was stirred at rt for 10 min and allowed to settle. The clear yellow supernatant liquid was drawn by a syringe and was used in the following reaction.

The aftermentioned clear yellow solution of Wittig reagent (114 µL, 0.011 mmol, 7 equiv) was taken in a 5 mL vial and cooled to 0 ºC. To this solution was added a solution of the ketone **1.161** in 100 µL THF (1.9 mg, 0.0016 mmol, 1 equiv) dropwise via syringe and rinsed with THF (50 µL). The initial clear yellow solution in the vial turned into a white ppt. After stirring for 10 min at 0 ºC, the reaction was quenched by addition of pH 7 buffer (2 mL) and diluted with 20% EtOAc/hexanes (5 mL). The phases were separated and the aqueous phase was extracted with 20% EtOAc/hexanes (3 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography in a pipette column (0.5 x 6 cm) using 10% EtOAc/hexanes collecting 6 x 50 mm fractions. The fractions from 6-12 provided the desired product **1.162** (1.9 mg, 70%,) as a colorless liquid. R_f = 0.42 (20% EtOAc/hexanes); Analytial data: $[\alpha]_D^{20} = +7$ (*c* = 0.1, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.72-7.70 (m, 4H), 7.45-7.24 (m, 11H), 6.19-6.15 (m, 2H), 5.99 (d, *J* = 1.4 Hz,, 1H), 5.77 (d, *J* = 15.1 Hz, 1H), 5.64-5.60 (m, 1H), 5.30 (dd, *J* = 15.6, 8.3 Hz, 1H), 5.23 (s, 1H), 4.93 (d, *J* = 6.8 Hz, 1H), 4.85 (d, *J* = 6.8 Hz, 1H), 4.83 (s, 2H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.68 (d, *J* = 11.7 Hz, 1H), 4.55 (s, 1H), 4.53-4.4.50 (m, 1H), 4.41 (s, 1H), 4.22-4.20 (m, 1H), 3.85 (ddd, *J* = 11.5, 8.9, 2.2 Hz, 1H), 3.75-3.70 (m, 1H),

3.69 (s, 3H), 3.20 (t, J = 8.7, 1H), 3.09 (s, 3H), 2.52 (dd, J = 15.1, 9.7 Hz, 1H), 2.42 (dd, J = 15.1, 2.4 Hz, 1H), 2.38-1.98 (m, 9H), 1.82 (t, J = 13.1 Hz, 1H), 1.78 (t, J = 11.2 Hz, 1H), 1.56-1.29 (m, 8H), 1.25 (s, 3H), 1.18 (d, J = 6.3, 1H), 1.10 (s, 3H), 1.06 (s, 3H), 0.98 (s, 9H), 0.92 (t, J = 7.3, 3H), 0.86 (s, 3H), 0.79 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 171.1, 167.0, 165.6, 153.2, 151.8, 146.7, 145.9, 144.5, 142.6, 138.1, 136.2 (x2), 136.0, 135.1, 133.6, 129.8, 129.7, 128.6, 128.5, 128.2, 127.9, 127.7, 125.5, 119.4, 118.7, 110.0, 109.1, 106.3, 103.4, 93.7, 82.4, 81.3, 76.5, 73.7, 72.2, 70.9 (x2), 70.1, 69.7, 67.2, 53.0, 51.3, 48.9, 45.3, 44.1, 41.4, 40.6, 39.2, 38.7, 35.2, 33.5, 31.1, 29.9, 27.0, 25.8, 22.3, 22.0, 20.1 (x2), 19.5, 14.8, 13.9; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 53.0, 51.3, 27.0, 25.8, 22.3, 20.1 (x2), 14.8, 13.9; CH_2 δ 109.1, 106.3, 93.7, 69.7, 45.3, 44.1, 41.4, 40.7, 39.2, 38.7, 35.2, 33.6, 31.1, 22.0; CH_1 δ 146.7, 145.8, 142.4, 136.2 (x2), 136.0, 129.8, 129.7, 128.6 (x2), 128.2, 127.9, 127.7, 125.6, 119.4, 118.7, 82.4, 81.3, 77.2, 76.5, 73.7, 72.2, 70.9, 70.1, 67.2; CH_0 δ; 171.7, 167.0, 165.6, 153.2, 151.8, 144.5, 138.1, 135.1, 133.6, 110.0, 103.4, 29.9, 19.5; IR (neat) 2954, 2857, 1652, 1461, 1348, 1253, 1178, 1083, 933, 836, 776 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{69}H_{92}NaO_{13}Si$(M+Na): 1179.6205, found: 1179.6216.



Preparation of Merle 32: To a stirring solution of the BPS ether **1.162** (1.9 mg, 0.0016 mmol, 1.0 equiv) in a 5:4:1 THF/MeOH/ pyridine solution (800 µL, 0.002M) at 0 ºC in a 2 mL plastic centrifuge tube was added

HF·Py (20 %, 400 µL). The reaction mixture was stirred at 0 ºC for 30 min and then warmed to rt. Stirring was continued for 48 h and the reaction mixture was then quenched by pipetting into a mixture of saturated aqueous $NaHCO_3$ solution and EtOAc (5 mL each). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 5 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was taken to the next step without further purification.

To a 4 mL reaction vial containing the analogue precursor from the previous reaction was added a 0.25 M solution of $LiBF_4$ in 25:1 CH_3CN/ H_2O (300 µL, 0.073 mmol, 45.0 equiv). The reaction vial was sealed and the mixture was allowed to stir at 80 ºC for 16 h. After cooling to rt, the reaction mixture was diluted with EtOAc (5 mL) and was quenched with saturated aqueous $NaHCO_3$ solution (5 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated. Purification was accomplished using flash column chromatography with a 0.5 x 6 cm silica gel column, eluting with 20% EtOAc/hexanes (10 mL), collecting 6 x 50 fractions followed by 50% EtOAc/hexanes. The product containing fractions (15-29) were combined and concentrated under reduced pressure to provide Merle 32 (0.8 mg, 61% over 2 steps) as white powder: R_f = 0.57 (50% EtOAc/hexanes; $[\alpha]_D^{20}$ = -3 (*c* = 0.08, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 6.18-6.16 (m, 2H), 6.02 (d, *J* = 1.9 Hz, 1H), 5.81 (d, *J* = 15.1Hz, 2H), 5.77 (d, *J* = 16.1 Hz, 1H), 5.33 (dd, *J* = 15.6, 8.3 Hz, 1H), 5.26 (s, 1H), 5.21 (s, 1H), 4.73 (dd, *J* = 6.4, 1.5 Hz, 1H), 4.71 (s, 1H), 4.66 (s, 1H), 4.48 (d, *J* = 11.7 Hz, 1H), 4.26-4.22 (m, 1H), 4.09-4.00 (m, 2H), 3.82 (dd, *J* = 12.2, 5.8 Hz, 1H), 3.71 (dd, *J* = 8.7, 2.4

Hz, 1H), 3.68 (s, 3H), 3.51 (t, *J* = 11.7Hz, 1H), 3.44 (ddd, *J* = 11.0, 5.9, 1.7 Hz, 1H), 3.12 (dd, *J* = 11.4, 1.7 Hz, 1H), 2.49 (dd, *J* = 12.0, 1.8 Hz, 1H), 2.45-2.41 (m, 1H), 2.28 (t, *J* = 13.1 Hz, 1H), 2.18-1.90 (m 12H), 1.83 (ddd, *J* = 14.0, 11.6, 2.7 Hz, 2H), 2.06 (s, 3H), 1.80-1.74 (m, 2H), 1.50-1.43 (m, 3H), 1.24 (d, *J* = 6.8 Hz, 3H), 1.15 (s, 3H), 1.01 (s, 6H), 0.99 (s, 3H), 0.92 (t, *J* = 7.3 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.3, 167.2, 165.7, 152.6, 152.2, 146.4, 145.6, 144.1, 138.5, 130.3, 128.6, 119.7, 118.9, 108.6, 106.7, 99.2, 86.3, 80.0, 78.0, 74.3 (x2), 73.8, 73.3, 70.5, 70.4, 68.9, 64.8 (x2), 45.0, 43.0, 42.7, 41.5, 40.5, 39.7, 37.1, 36.0, 35.2, 31.5, 24.8, 22.8, 22.0, 20.7, 20.0, 13.9 ; IR (neat) 2928, 1778, 1721, 1659, 1454, 1368, 1214, 1073, 837, 757, 701 cm^{-1}; HRMS calcd for $C_{44}H_{64}O_{12}Na$ (M+Na): 807.4295, found: 807.4300.

Biological Experiments and Data for Merle 32

[^{3}H]PDBu binding assay. The binding assay of Mele 32 was done similar to that of Merle 30. The K_i for Merle 30 was found to be 1.08 nM. nM (average of three determinations).

Attachment and cell proliferation of U937 and LNCaP cells. The attachment and proliferation of U937 and LNCaP cells were performed similar to that of Merle 30 and the results are shown in Figure **1.76** and Figure **1.77**.

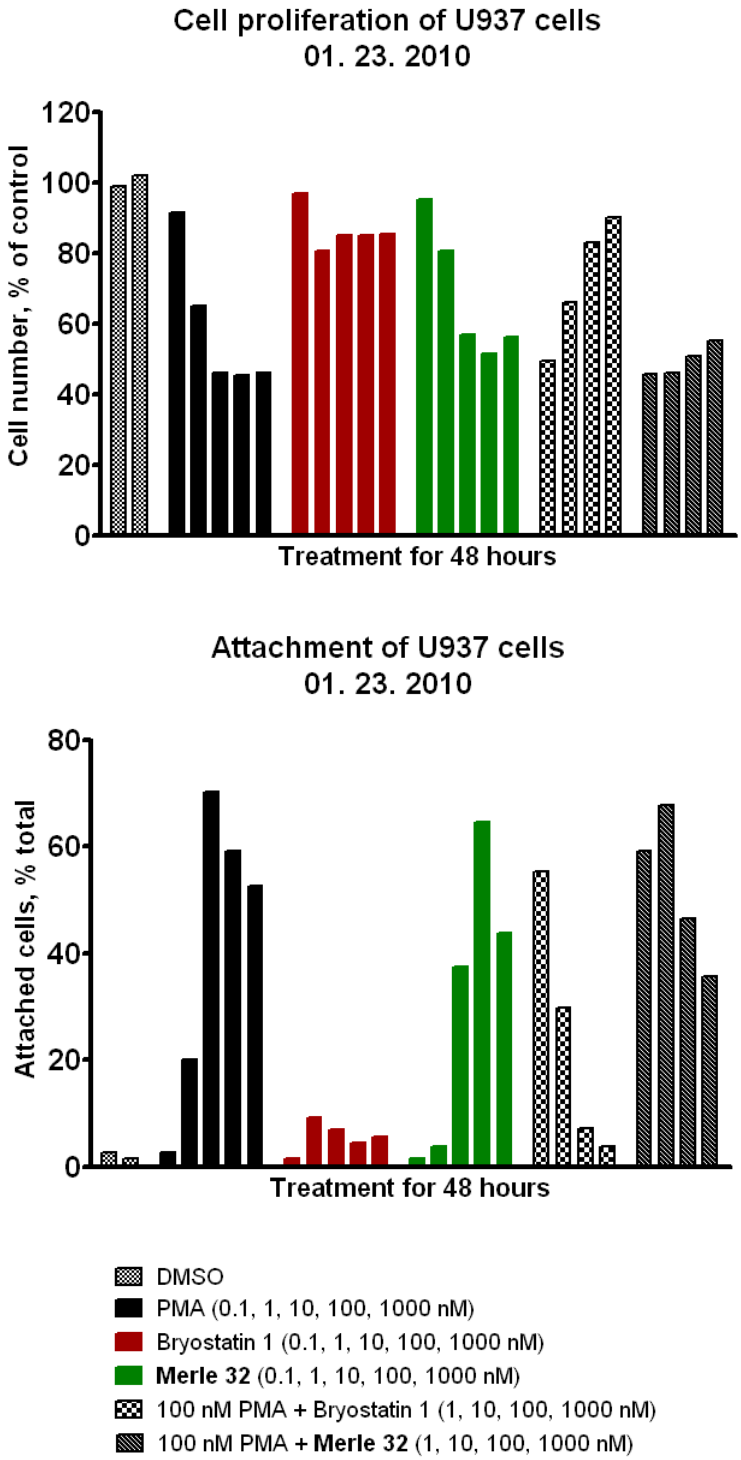


Figure 1.76. Proliferation (top) and Attachment (bottom) of U937 Cells by Merle 32

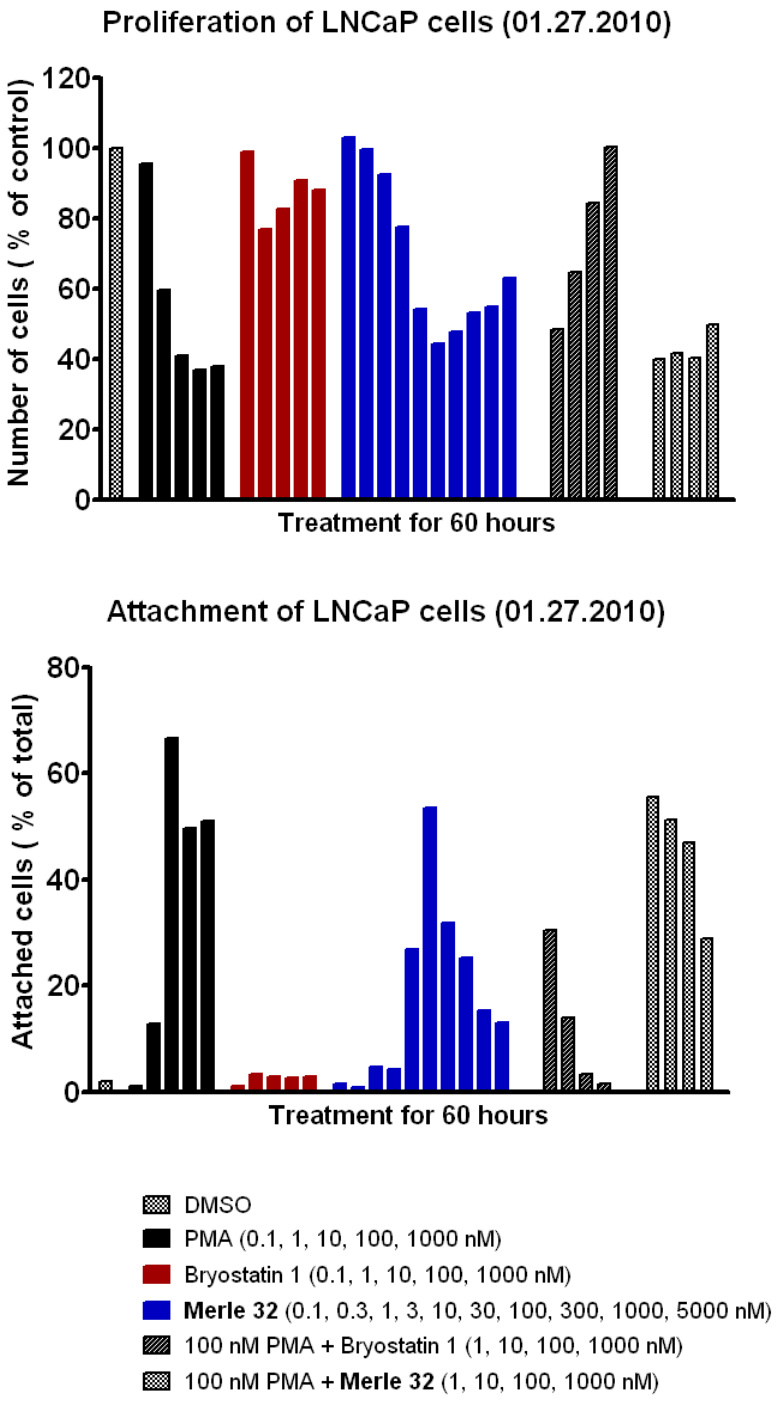


Figure 1.77. Assays Involving LNCaP Cell Lines

References

1. Pettit, G. R.; Day, J. F.; Hartwell, J. L.; Wood, H. B. *Nature* **1970,** *227*, 962-963.

2. Pettit, G. R.; Herald, C. L.; Doubek, D. L.; Herald, D. L.; Arnold, E.; Clardy, J. *J Am. Chem. Soc.* **1982,** *104*, 6846-6848.

3. Lopanik, N.; Gustafson, K. R.; Lindquist, N. *J. Nat. Prod.* **2004**, *67,* 1412-1414.

4. Lopanik, N.; Lindquist, N.; Targett, N. *Oecologia* **2004,** 139, 131-139.

5. (a) Pettit, G. R.; Herald, C. L.; Kamano, Y.; Gust, D.; Aoyagi, R. *J. Nat. Prod.* **1983,** *46*, 528-531; (b) Pettit, G. R.; Herald, C. L.; Kamano, Y. *J. Org. Chem.*, **1983,** *48*, 5354-5356; (c) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tozawa, M. *J. Am. Chem. Soc.* **1984,** *106*, 6768-6771; (d) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tozawa, M. *Can. J. Chem.* **1985,** *63*, 1204-1204; (e) Pettit, G. R.; Kamano, Y.; Aoyagi, R.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Rudloe, J. J., *Tetrahedron* **1985,** *41*, 985-994; (f) Pettit, G. R.; Kamano, Y.; Herald, C. L. *J. Nat. Prod.* **1986,** *49*, 661-664; (g) Pettit, G. R.; Kamano, Y.; Herald, C. L. *J. Org. Chem.* **1987,** *52*, 2848-2854; (h) Pettit, G. R.; Leet, J. E.; Herald, C. L.; Kamano, Y.; Boettner, F. E.; Baczynskyj, L.; Nieman, R. A. *J. Org. Chem.* **1987,** *52*, 2854-2860; (i) Pettit, G. R.; Gao, F.; Sengupta, D.; Coll, J. C.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Van Camp, J. R.; Rudloe, J. J.; Nieman, R. A. *Tetrahedron* **1991,** *47*, 3601-3610; (j) Pettit, G. R.; Gao, F.; Blumberg, P. M.; Herald, C. L.; Coll, J. C.; Kamano, Y.; Lewin, N. E.; Schmidt, J. M.; Chapuis, J.-C. *J. Nat. Prod.* **1996,** *59* (3), 286-289.

5. Pettit, G. R.; Gao, F.; Herald, D. L.; Blumberg, P. M.; Lewin, N. E.; Nieman, R. A. *J. Am. Chem. Soc.***1991,** *113*,6693-6695.

6. (a) Koutcher, J. A.; Motwani, M.; Zakian, K. L.; Li, X.-K.; Matei, C.; Dyke, J. P.; Ballon, D.; Yoo, H.-H.; Schwartz, G. K. *Clin. Cancer Res.* **2000**, *6*, 1498-1507. (b) Mohammad, R. M.; Wall, N. R.; Dutcher, J. A.; Al-Katib, A. M. *Clin. Cancer Res.* **2000**, *6*, 4950–4956. (c) El-Rayes, B. F.; Gadgeel, S.; Shields, A. F.; Manza, S.; Lorusso, P.; Philip, P. A. *Clin. Cancer Res.* **2006**, *12*, 7059–7062. (d) Basu, A.; Lazo, J. S. *Cancer Res.* **1992**, *52*, 3119-3124.

7. Hess, A. D.; Silanskis, M. K.; Esa, A. H.; Pettit, G. R.; May, W. S. *J. Immunol*., **1988**, *141*, 3263-3269.

8. Mehla, R.; Bivalkar-Mehla, S.; Zhang, R.; Handy, I.; Albrecht, H.; Giri, S.; Nagarkatti, P.; Nagarkatti, M.; Chauhan, A. *PLoS One*. **2010**, 5, e11160.

9. (a) Sun, M.-K.; Alkon, D. L. *Eur. J. Pharmacol.* **2005**, *512*, 43-51. (b) Alkon, D. L.; Epstein, H.; Kuzirian, A.; Bennett, M. C.; Nelson, T. J. *Proc. Natl. Acad. Sci.*

U.S.A. **2005**, *102*, 16432–16437. (c) Wang, D.; Darwish, D. S.; Schreurs, B. G.; Alkon, D. L., *Behav.Pharmacol.* **2008**, 19, 245-256.

10. (a) Etcheberrigaray, R.; Tan, M.; Dewachter, I.; Kuiperi, C.; Van der Auwera, I.; Wera, S.; Qiao, L.; Bank, B.; Nelson, T. J.; Kozikowski, A. P.; Van Leuven, F.; Alkon, D. L. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11141–11146. (b) Alkon, D. L.; Sun, M.-K.; Nelson, T. J. *Trends Pharmacol. Sci.* **2007**, *28*, 51-60.

11. For clinical trials, see: http://www.brni.org/news/Press Release42209.htm.

12. Sun, M.-K.; Hongpaisan, J.; Nelson, T. J.; Alkon, D. L. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 13620-13625.

13. Kasanietz, M. G.; Lewin, N. E.; Gao, F.; Pettit, G. R.; Blumberg, P. M. *Mol. Pharmacol.* **1994**, *46*, 374-379.

14. Reyland, M. E.; Insel, P. A.; Messing, R. O.; Dempsey, E. C.; Newton, A. C.; Mochly-Rosen, D.; Fields, A. P. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2000**, *279*, 429-438. (b) Griner, E. M.; Kazanietz, M. G. *Nat. Rev. Cancer* **2007**, *7*, 281-294. (c) Roffey, J.; Rosse, C.; Linch, M.; Hibbert, A.; McDonald, N. Q.; Parker, P. J. *Current Opinion in Cell Biology* **2009**, *21*,1-12.

15. Newton, A. C. *Chem. Rev.* **2001**, *101*, 2353-2364.

16. Brose, N.; Rosenmund, C. *J. Cell Sci.* **2002**, *115*, 4399-4411.

17. Keranen, L. M.; Dutil, E. M.; Newton, A. *Curr. Biol.* **1995**, *5*, 1394-1403.

18. Smith, J. B.; Smith, L.; Pettit, G. R, Biochem. *Biophys. Res. Comm.* **1985**, *132*, 939-945.

19. Blumberg, P. M., *Mol. Carcinog.* **1991**, *4*, 339-344

.

20. Blumberg, P. M., Pettit, G. R. in New Leads and Targets in Drug Research, Munksgaard, Copenhagen, **1992**, 273-285.

21. Hennings, H.; Blumberg, P. M.; Pettit, G. R.; Herald, C. L.; Shores, R.; Ysupa, S. H. *Carcinogenesis* **1987**, *8*, 1343-1346.

22. D. E. Schaufelberger, M. P. Koleck, J. A. Beutler, A. M. Vatakis, A. B. Alvarado, P. Andrews, L. V. Marzo, G. M. Muschik, J. Roach, J. T. Ross,W. B. Lebherz, M. P. Reeves, R. M. Eberwein, L. I. Rodgers, R. P. Testerman, K. M. Snader, S. Forenza, *J. Nat. Prod.* **1991**, 54, 1265-1270.

23. Mendola, D. *Biomol. Eng.* **2003**, *11*, 1543-1542.

24. Hildebrand, M.; Waggoner, L. E.; Liu, H.; Sudek, S.; Allen, S.; Anderson, C.; Sherman, D. H.; Haygood, M. G. *Chem. Biol.* **2004**, *11*, 1543-1552.

25. Sudek, S.; Lopanik, N. B.; Waggoner, L. E.; Hildebrand, M.; Anderson, C.; Liu, H.; Patel, A.; Sherman, D. H.; Haygood, M. G. *J. Nat. Prod.* **2007**, **70**, 67-74.

26. Kageyama, M.; Tamura, T.; Nantz, M. H.; Roberts, J. C.; Somfai, P.; Whritenour, D. C.; Masamune, S. *J. Am. Chem. Soc.* **1990**, *112*, 7407-7408.

27. Evans, D. A.; Carter, P. H.; Carreira, E. M.; Charette, A. B.; Prunet, J. A.; Lautens, M. *J. Am. Chem. Soc.* **1999**, *121*, 7540-7552.

28. Ohmori, K.; Ogawa, Y.; Obitsu, T.; Ishikawa, Y.; Nishiyama, S.; Yamamura, S. *Angew. Chem. Int. Ed.* **2000**, *39*, 2290-2294.

29. Trost, B. M.; Dong, G. *Nature* **2008**, *456*, 485-488.

30. (a) Wender, P. A.; Cribbs, C. M.; Koehler, K. F.; Sharkey, N. A.; Herald, C. L.; Kamano, Y.; Pettit, G. R.; Blumberg, P. M. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85*, 7197-7201. (b) Wender, P. A.; Debrabander, J.; Harran, P. G.; Jimenez, J.-M.; Koehler, M. F. T.; Lippa, B.; Park, C.-M.; Siedenbiedel, C.; Pettit, G. R. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 6624-6629.

31. Wender, P. A.; De Brabander, J.; Harran, P. G.; Jimenez, J.-M.; Koehler, M. F. T.; Lippa, B.; Park, C.-M.; Shiozaki, M. *J. Am. Chem. Soc.* **1998**, *120*, 4534-4535.

32. Wender, P. A.; Baryza, J. L.; Bennett, C. E.; Bi, F. C.; Brenner, S. E.; Clarke, M. O.; Horan, J. C.; Kan, C.; Lacote, E.; Lippa, B.; Nell, P. G.; Turner, T. M. *J. Am. Chem. Soc.* **2002**, *124*, 13648-13649.

33. Wender, P. A.; DeChristopher, B. A.; Schrier, A. J. *J. Am. Chem. Soc.* **2008**, *130*, 6658-6659.

34. (a) Wender, P. A.; De Brander, J.; Harran, P. G.; Hinkle, K. W.; Lippa, B.; Pettit, G. R. *Tetrahedron Lett.* **1998**, *39*, 8625-8628. (b) Wender, P. A.; Hinkle, K. W.; Koehler, M. F. T.; Lippa, B. *Med. Res. Rev.* **1999**, *19*, 388-407. (c) Wender, P. A.; Lippa, B.; Park, C.-M.; Irie, K.; Nakahara, A.; Ohigashi, H. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1687-1690. (d) Wender, P. A.; Lippa, B. *Tetrahedron Lett.* **2000**, *41*, 1007-1011. (e) Wender, P. A.; Hinkle, K. W. *Tetrahedron Lett.* **2000**, *41*, 6725-6729. (f) Wender, P. A.; Mayweg, A. V. W.; VanDeusen, C. L. *Org. Lett.* **2003**, *5*, 277-279. (g) Wender, P. A.; Koehler, M. F. T.; Sendzik, M. *Org. Lett.* **2003**, *5*, 4549-4552. (h) Baryza, J. L.; Brenner, S. E.; Craske, M. L.; Meyer, T.; Wender, P. A. *Chem. Biol.* **2004**, *11*, 1261-1267. (i) Wender, P. A.; Baryza, J. L.; Brenner, S. E.; Clarke, M. O.; Craske, M. L.; Horan, J. C.; Meyer, T. *Curr. Drug Discovery Technol.* **2004**, *1*, 1-11. (j) Stone, J. C.; Stang, S. L.; Zheng, Y.; Dower, N. A.; Brenner, S. E.; Baryza, J. L.; Wender, P. A. *J. Med. Chem.* **2004**, *47*, 6638-

6644. (k) Wender, P. A.; Hilinski, M. K.; Mayweg, A. V. W. *Org. Lett.* **2005**, *7*, 79-82. (l) Wender, P. A.; Clarke, M. O.; Horan, J. C. *Org. Lett.* **2005**, *7*, 1995-1998. (m) Wender, P. A.; Baryza, J. L. *Org. Lett.* **2005**, *7*, 1177-1180. (n) Wender, P. A.; Horan, J. C.; Verma, V. A. *Org. Lett.* **2006**, *8*, 5299-5302. (o) Wender, P. A.; Horan, J. C. *Org. Lett.* **2006**, *8*, 4581-4584. (p) Wender, P. A.; Verma, V. A. *Org. Lett.* **2006**, *8*, 1893-1896. (q) Wender, P. A.; Verma, V. A. *Org. Lett.* **2008**, *10*, 3331-3334.

35. Wender, P. A.; Baryza, J. L.; Hilinski, M. K.; Horan, J. C.; Kan, C.; Verma, V. A. *Drug Discovery Res.* **2007**, 127-162.

36. Keck, G. E.; Covel, J. A.; Schiff, T.; Yu, T. *Org. Lett.* **2002**, *4*, 1189-1192.

37. (a) Keck, G. E.; Truong, A. P. *Org. Lett.* **2005**, *7*, 2149-2152. (b) Keck, G. E.; Truong, A. P. *Org. Lett.* **2005**, *7*, 2153-2156.

38. Sanchez, C. C., Ph. D. Thesis, University of Utah, 2005.

39. Vrana, J. A.; Saunders, A. M.; Chellappan, S. P.; Grant, S. *Differentiation* **1998**, *63*, 33-42.

40. Keck, G. E.; Kraft, M. B.; Truong, A. P.; Li, W.; Sanchez, C. C.; Kedei, N.; Lewin, N.; Blumberg, P. M. *J. Am. Chem. Soc.* **2008**, *130*, 6660-6661.

41. Keck, G. E.; Welch, D. S.; Poudel, Y. B. *Tetrahedron Lett.* **2006**, *47*, 8267-8270.

42. Mancuso, A. J.; Brownfain, D. S.; Swern, D., *J. Org. Chem.*, **1978**, *44*, 4148-4150.

43. (a) Keck, G. E.; Tarbet, K. H.; Geraci, L. S. *J. Am. Chem. Soc.* **1993**, *115*, 8467-8468. (b) Keck, G. E.; Krishnamurthy, D. *Org. Synth.* **1998**, *75*, 12-16.

44. Parikh, J. R.; Doering, W. v. E. *J. Am. Chem. Soc.* **1967**, *89*, 5505-5507.

45. Hirai, K.; Ooi, H.; Esumi, T; Iwabuchi, Y.; Hatakeyama, S. *Org. Lett.* **2003**, *5*, 857-859.

46. Keck, G. E.; Welch, D. S.; Vivian, P. K. *Org. Lett.* **2006**, *8*, 3667-3670.

47. Evans, D. A.; Allison, B. A.; Yang, M. G.; Masse, C. E. *J. Am. Chem. Soc.* **2001**, *123*, 10840-10852.

48. (a) Keck, G. E.; Boden, E. P. *Tetrahedron Lett.* **1984**, *25*, 265-268. (b) Keck, G. E.; Boden, E. P. *Tetrahedron Lett.* **1984**, *25*, 1879-1882.

49. (a) Keck, G. E.; Abbott, D. E. *Tetrahedron Lett.* **1984**, *25*, 1883-1886. (b) Keck, G. E.; Castellino, S.; Wiley, M. R. *J. Org. Chem.* **1986**, *51*, 5478-5480. (c) Keck, G. E.; Castellino, S. *J. Am. Chem. Soc.* **1986**, *108*, 3847-3849.

50. Cuny, G. D.; Buchwald, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 2066-2068.

51. Liu, H. J.; Rose, P. A.; Sasaki, D. J. *Can. J. Chem.* **1991**, *69*, 934-936.

52. (a) Keck, G. E.; Li, X.-Y.; Krishnamurthy, D. *J. Org. Chem*. **1995**, *60*, 5998-5999. (b) Keck, G. E.; Krishnamurthy, D. *Synth. Commun*. **1996**, *26*,367-378.

53. Noyori, R.; Tomino, I.; Tanimoto, Y.; Nishizawa, M., *J. Am. Chem. Soc.* **1984**, *106*, 6709-6716.

54. Corey, E. J.; Shibata, S.; Bakshi, R., K. *J. Chem. Soc., Chem. Commun.* **1988**, *8*, 469-470.

55. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512-519.

56. Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *J. Chem. Soc., Chem. Commun.* **1987**, 1625-1627.

57. (a) Luche, J. L. *J. Am. Chem. Soc.* **1978**, *100*, 2226-2227. (b) Luche, J. L.; Rodriguez-Hahn, L.; Crabbe, P. *J. Chem. Soc., Chem. Commun.* **1978**, 601-602.

58. Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989-1993.

59. Lipshutz, B. H.; Harvey, D. F. *Synth. Commun.*, **1982**, *12*, 267-277.

60. Keck, G. E.; Poudel, Y. B.; Welch, D. S.; Kraft, M. B.; Truong, A. P.; Stephens, J. C.; Kedei, N.; Lewin, N. E.; Blumberg, P. M. *Org. Lett.* **2009**, *11*, 593-596.

61. Keck, G. E.; Li, W;; Kraft, M. B; Kedei, N.; Lewin, N. E.; Blumberg, P. M. *Org. Lett.* **2009**, *11*, 2277-2280.

62. Kimura, K.; Mizutani, M. Y.; Tomioka, N.; Endo, Y.; Shudo, K.; Itai, A. *Chem. Pharm. Bull.* **1999**, *47*, 1134-1137.

63. Chatterjee, A. K.; Choi, T. L.; Sanders, D. P.; Grubbs, R. H. *J. Am. Chem. Soc.* **2003**, *125*, 11360-11370.

64. (a) Tanaka, K.; Ohta, Y.; Fuji, K. *Tetrahedron Lett.* **1993**, *34*, 4071-4074.

65. Baeyer, A.; Villiger, V., *Chem. Ber*., **1899**, *32*, 3625-3633.

66. Keck, G. E.; Poudel, Y. B.; Rudra, A.; Stephens, J. C.; Kedei, N.; E. Lewin, N. E.; Peach, M. L.; Blumberg, P. M. *Angew. Chem. Int. Ed.* **2010**, *49*, 4580-4584.

67. (a) Gonzalez-Guerrico, A. M.; Meshki, J.; Xiao, L.; Benavides, F.; Conti, C. J.; Kazanietz, M. G., *J. Biochem. Mol. Biol.*, **2005**; 38, 639-645.(b) Gschwend, J., E.; Fair, W. R.; Powell, C., T., *Mol. Pharmacol.*, **2000**, 57, 1224-1234.

68. Petasis, N., A.; Bzowej, E., I. *J. Am. Chem. Soc.*, **1990**, *112*, 6392-6394.

69. Wittig, G.; Haag, W. *Chem. Ber.* **1955**, *88*, 1654-1666.

70. Tebbe, F. N.; Parshall, G. W.; Reddy, G. S. *J. Am. Chem. Soc.* **1978**, *100*, 3611-3613.

71. Hocevar, B. A.; Morrow, D. M.; Tykocinski, M. L.; Fields, A. P. *J. Cell Sci.* **1992**, *101*, 671-679.

72. Wang, Q. J.; Bhattacharyya, D.; Garfield, S.; Nacro, K.; Marquez, V. E.; Blumberg, P. M. *J. Biol. Chem.* **1999**, *274*, 37233-37239.

73. Armarego, W. L. F.; Perrin, D. D., *Purification of Laboratory Chemicals, Fourth Edition*. Butterworth-Heinemann: Oxford, **1997**.

74. Watson, S. C.; Eastham, J. F. *J. Organomet. Chem.* **1967**, *9*, 165-168.

75. Lewin, N. E.; Blumberg, P. M. *Methods Mol. Biol.* **2003**, *233*, 129-156.

76. Sundstrom, C.; Nilsson, K. "*Int. J. Cancer* **1976**, *17*, 565-577.

77. Zhang, G. G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H. *Cell* **1995**, *81*, 917-924.

CHAPTER 2

THE TOTAL SYNTHESIS OF BRYOSTATIN 1

Introduction

Bryostatin 1 is the flagship member of a family of 20 macrolides that were isolated by Pettit and co-workers from the marine bryozoan *Bugula neritina*, after finding the antineoplastic activity of its extracts against murine P388 lymphocytic leukemia.[1] The gross structure of bryostatin 1 (Figure **2.1**) was determined in 1982 using NMR, IR, and mass spectrometry, whereas the absolute stereochemistry was determined using single crystal X-ray crystallography of bryostatin 1[2] and the C_7 *para*-bromo benzoate derivative

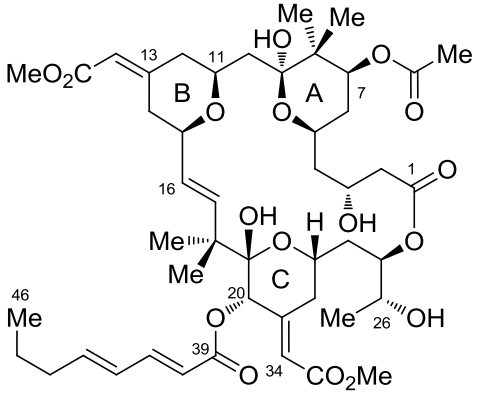


Figure 2.1. Structure of Bryostatin 1

of bryostatin 2.[3] Since the first isolation of bryostatin 1, nineteen other members of the family have been isolated and characterized.[4]

Bryostatin 1 is a highly oxygenated natural product that has a number of architecturally interesting features. The 20-membered macrolactone houses three embedded functionalized pyran rings often referred to as A-, B- and C-rings. The A- and the C-rings of the bryostatin 1 are more functionalized and are present in sensitive lactol forms. The B- and C-ring each have an unique exocyclic α,β-unsaturated methyl ester at 4 position. In addition, bryostatin 1 has a pair of gem-dimethyl group at the C_8 and C_{18} positions. The molecule has three olefins, a *trans* olefin in the macrocycle and a pair of *E* olefins on the octadieoante side chain on the C-ring. The three-dimensional structure of bryostatin 1 is held together by a network of three hydrogen bonds. The C_3 hydroxyl group serves as a hydrogen bold acceptor for C_{19} hydroxyl group as well as acts as a donor for a bifurcated hydrogen bond to the A- and B-ring pyran oxygens. Most of the bryostatins differ from each other at C_7 and C_{20} positions in which they have different ester substituents. One of the simplest members of the family is bryostatin 16 which has *t*-butyl acetate at the C_7 position and the C_{19}-C_{20} groups are replaced by an olefin. On the other hand, bryostatin 3, 19 and 20 have an additional five membered lactone fused with the C-ring.

Due to its promising anticancer activity, bryostatin 1 is in some 80 clinical trials for cancer chemotherapy.[5] Additionally, it has shown other impressive biological activities such as reversal of multidrug resistance,[6] synergetic effect with other anticancer agents,[7] stimulation of the immune system,[8] enhancement of memory,[9] activity against Alzheimer's disease,[10] and neutoprotective activity after stroke.[11] More recently,

bryostatin has been shown to have anti-HIV activity.[12] Although the mechanism of action of bryostatin 1 with respect to these biological activities is still under investigation, it is known to have an exceptionally high binding ability to Protein Kinase C (PKC) isozymes.[13]

Because of these remarkable biological activities, bryostatin 1 has potential as a therapeutic agent against numerous diseases. However, further clinical development of the natural product has been hampered by extremely low natural abundance. The largest isolation of the bryostatins from 28000 lbs of *Bugula neritina* provided only 18 grams of bryostatin 1 with a yield of 0.0014%.[14] As the isolation of the bryostatins from such non-renewable source has a harmful ecological impact, other sources of bryostatins have been explored. One of them is the aquaculture of the *Bubula neritina* by CalBiomarine Technologies.[15] However, the process has not been cost effective due to low production of bryostatins and has been abandoned. A study towards the biosynthesis of the bryostatins resulted in the isolation of its gene cluster and further development is still in its infancy.[16]

Previous Total Syntheses of Bryostatins

The challenging architecture, impressive biological profile and scarce natural abundance of bryostatins have prompted a number of research groups towards the total synthesis of these natural products. Despite the enormous effort in this area, only four completed total syntheses of bryostatins have been reported (Figure **2.2**). The first bryostatin to be synthesized was bryostatin 7 by Masamune[17] group in 1990 which was followed by Evans'[18] synthesis of bryostatin 2 in 1999. In 2000, Yamamura completed

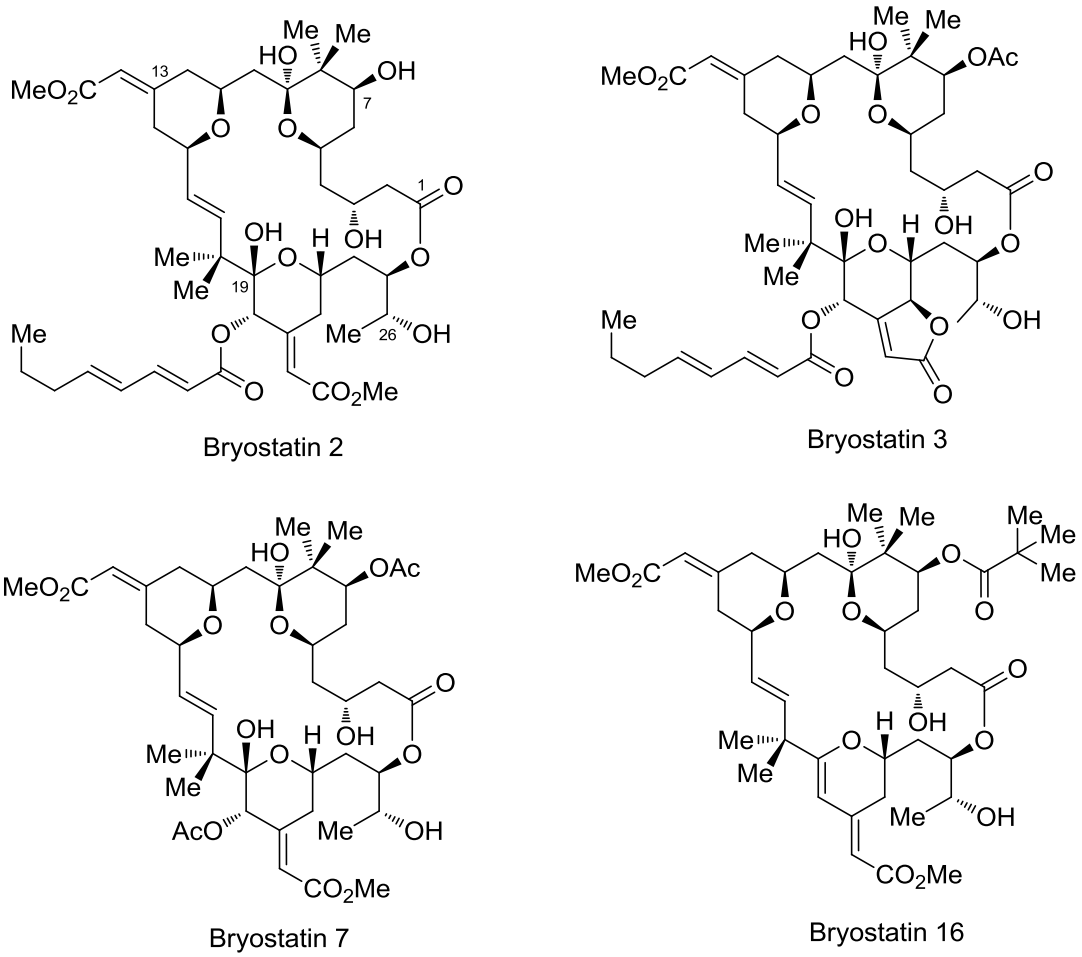


Figure 2.2. Structure of Synthesized Bryostatins

the synthesis of the most complex member of the family, bryostatin 3.[19] More recently, the Trost group finished the total synthesis of bryostatin 16 in 2002.[20] Moreover, Hale has reported the formal synthesis of bryostatin 7.[21] In addition, numerous fragments, partial syntheses and studies towards the syntheses have been disclosed.[22] This chapter will focus on the discussion of only the completed total syntheses of bryostatins and interested readers are directed to the reviews of bryostatin syntheses. [23]

Masamune's Total Synthesis of Bryostatin 7[17]

Bryostatin 7 was the first member of its family to be synthesized by Masamune in 1990. This synthesis is based on the extensive use of chiral boron enolate aldol chemistry developed earlier by Masamune. The synthetic plan involved a key C_{16}-C_{17} bond disconnection leading to a convergent union of A-B and C-ring fragments by Julia olefination which would be shared by most of the succeeding syntheses (Figure **2.3**). An intramolecular esterification would construct the macrocyclic lactone. This retrosynthesis thus divides the molecule horizontally into northern A-B-ring fragment and southern C-ring fragment. Both A and C pyran rings would be constructed from the ketalization of the corresponding keto alcohols where as B-ring would be formed by oxymercuration process. The linear A-B fragment would be formed by an asymmetric aldol reaction between the boron enolate of ketone **2.6** and aldehyde **2.5** whereas the all carbon skeleton of the C-ring **2.2** would be formed by an organometallic addition of iodide **2.8** to the aldehyde **2.7**.

The synthesis of the A-B-ring fragment commenced from aldehyde **2.10** which was prepared in three steps from commercially available 2,2-dimethylpropane-1,3-diol (Figure **2.4**). A two carbon homologation using Horner-Wadsworth-Emmons (HWE) reaction followed by DIBAL-H reduction of the ester provided an allylic alcohol **2.11** for the Sharpless asymmetric epoxidation (SAE) in the next step. The alcohol was oxidized and another two carbon homologation using Wittig reaction provided the α,β-unsaturated aldehyde **2.13**. Reduction of the aldehyde provided the allylic alcohol which was subjected to another SAE reaction providing the bisepoxide **2.14**. Both of the epoxides were opened using alcohol directed Red-Al reduction which installed C_5 and C_7

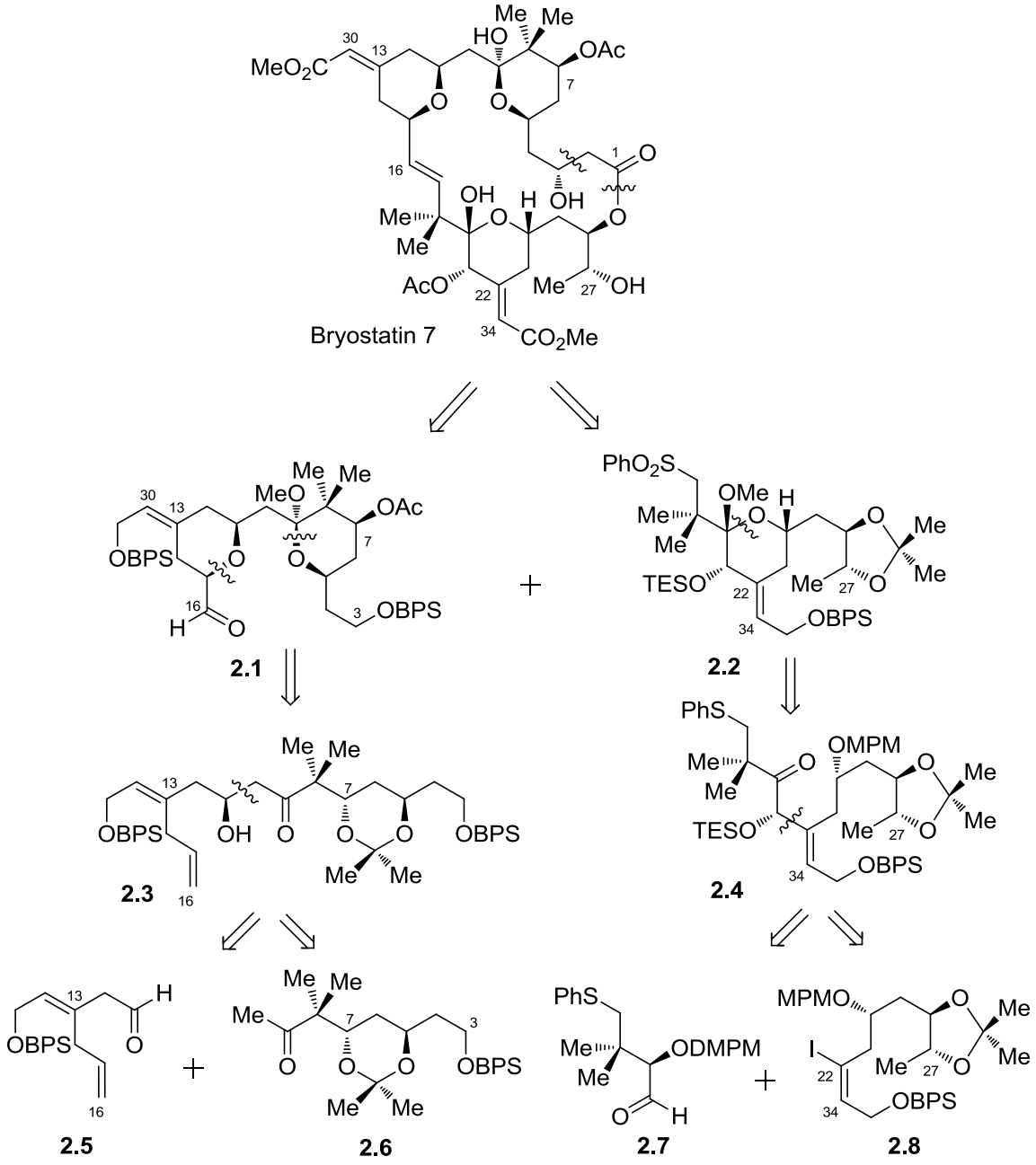


Figure 2.3. Masamune's Retrosynthesis of Bryoststin 7

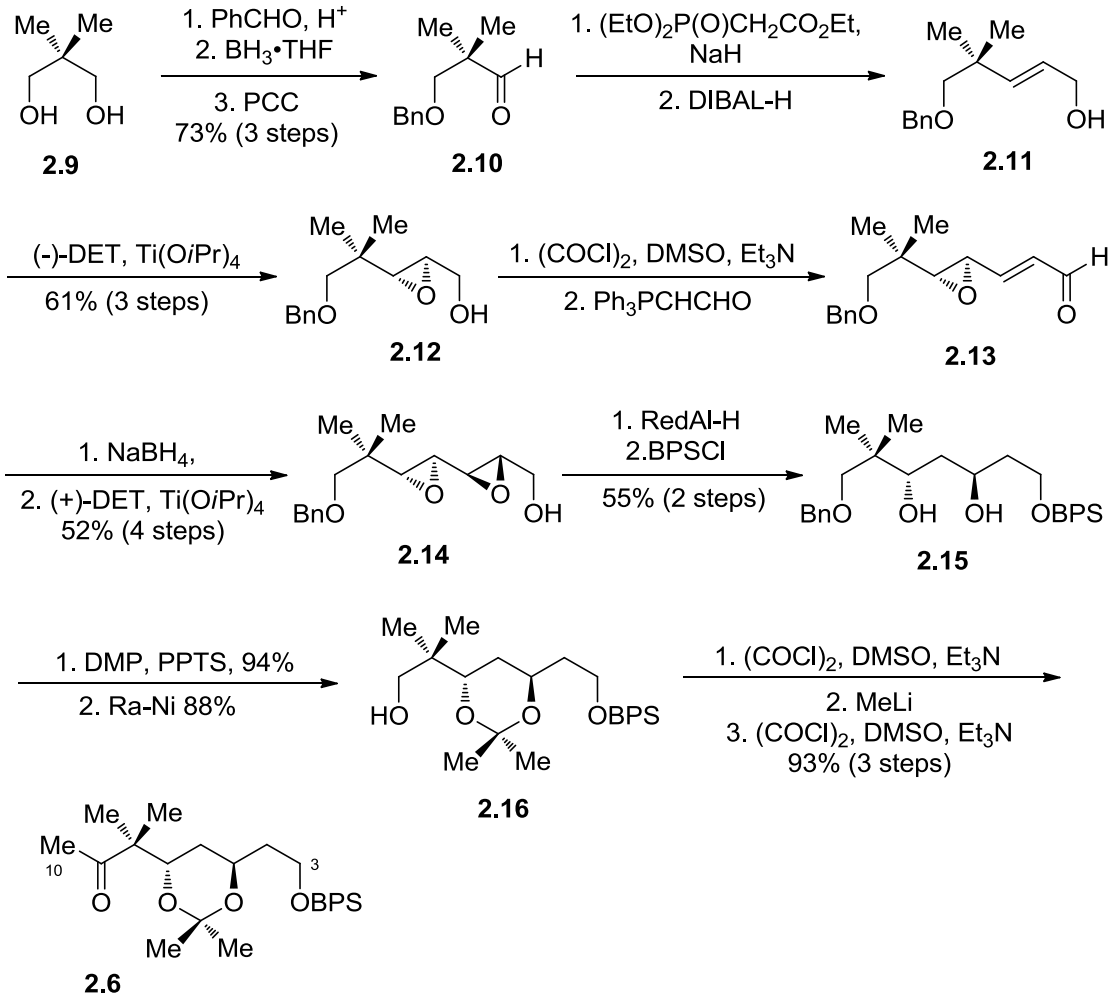


Figure 2.4. Preparation of C_3-C_{10} Subunit

stereocenters and the primary alcohol was selectively protected as BPS ether. The 1,3 diol was protected as acetonide which also verified their antistereochemical relationship. Deprotection of the benzyl group liberated the alcohol which was oxidized to aldehyde. Addition of the methyl lithium followed by the oxidation of resulting alcohol completed the synthesis of C_3-C_{10} fragment needed for the construction of A-B-ring. The aldehyde coupling partner (Figure **2.5**) for the aldol reaction was prepared from THP protected

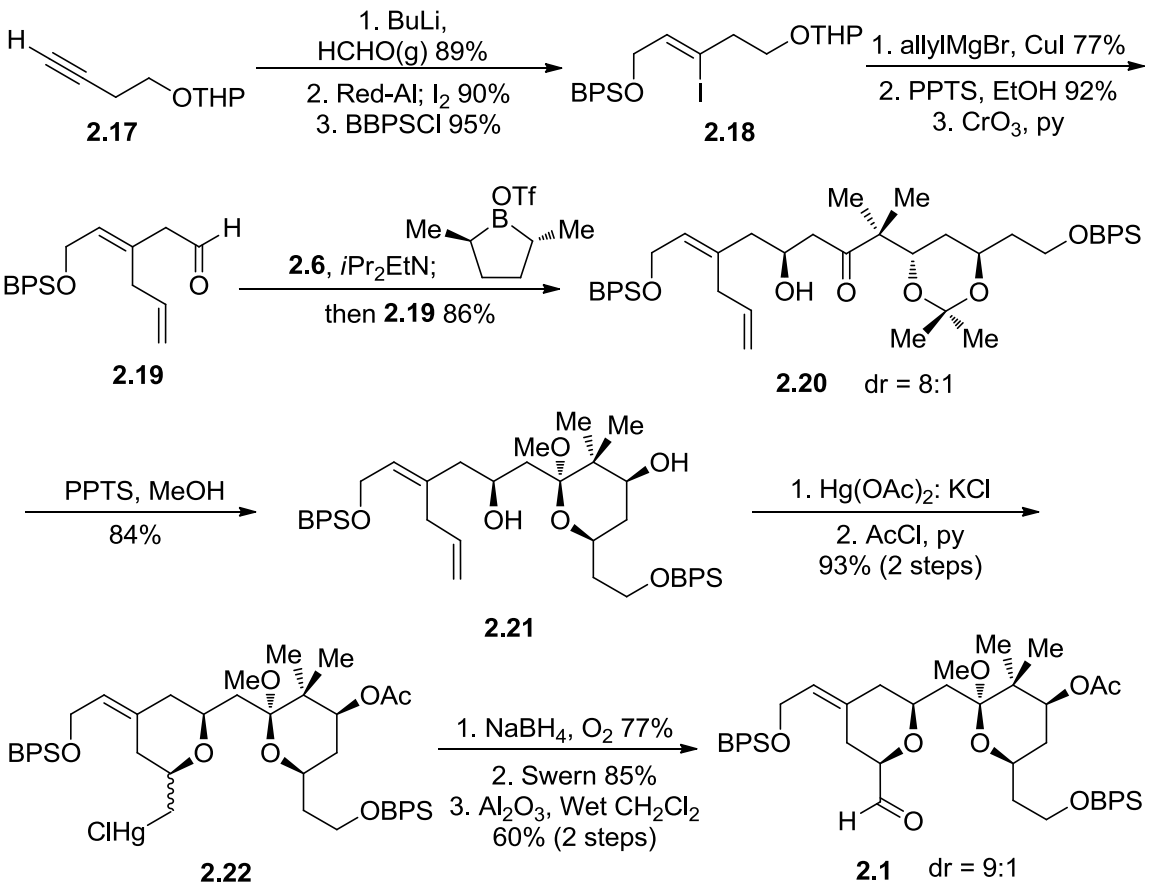


Figure 2.5. Masamune's Completion of C_1-C_{16} Fragment

propargyl alcohol **2.17**. Homologation using formaldehyde gave the primary alcohol which directed the Red-Al reduction of the alkyne and trapping it using iodine providedthe desired *Z* vinyl iodide. Coupling of the vinyl iodide with allylmagnesium bromide followed by the deprotection of the THP group and subsequent oxidation provided the aldehyde coupling partner **2.19**.

With both coupling partners in hand, enolization of the ketone **2.6** using chiral boron reagent followed by its addition to aldehyde **2.19** set the C_{11} sereocenter. Removal of the acetonide and insitu ketalization was carried out using PPTS/MeOH providing the

A-ring. In order to prepare B-ring, the precursor **2.21** was subjected to oxymercuration reaction giving an epimeric mixture at C_{15}. Demercuration of the intermediate **2.22** followed by Swern oxidation provided the epimeric aldehyde which was equilibrated to the desired cis pyran using wet alumina. This completed the synthesis of the A-B-ring unit in 24 longest linear steps starting from diol **2.9**.

The preparation of the C-ring subunit **2.7** began with carbamate formation followed by opening of the epoxide **2.23** (Figure **2.6**). The carbamate protecting group was swapped with the primary acetonide and the free secondary alcohol was protected as a DMP ether. Removal of the benzyl group followed by mesylation and displacement provided the pheyl sulfide **2.26**. An acidic removal of the acetonide followed by oxidative cleavage of diol provided the aldehyde partner **2.7**.

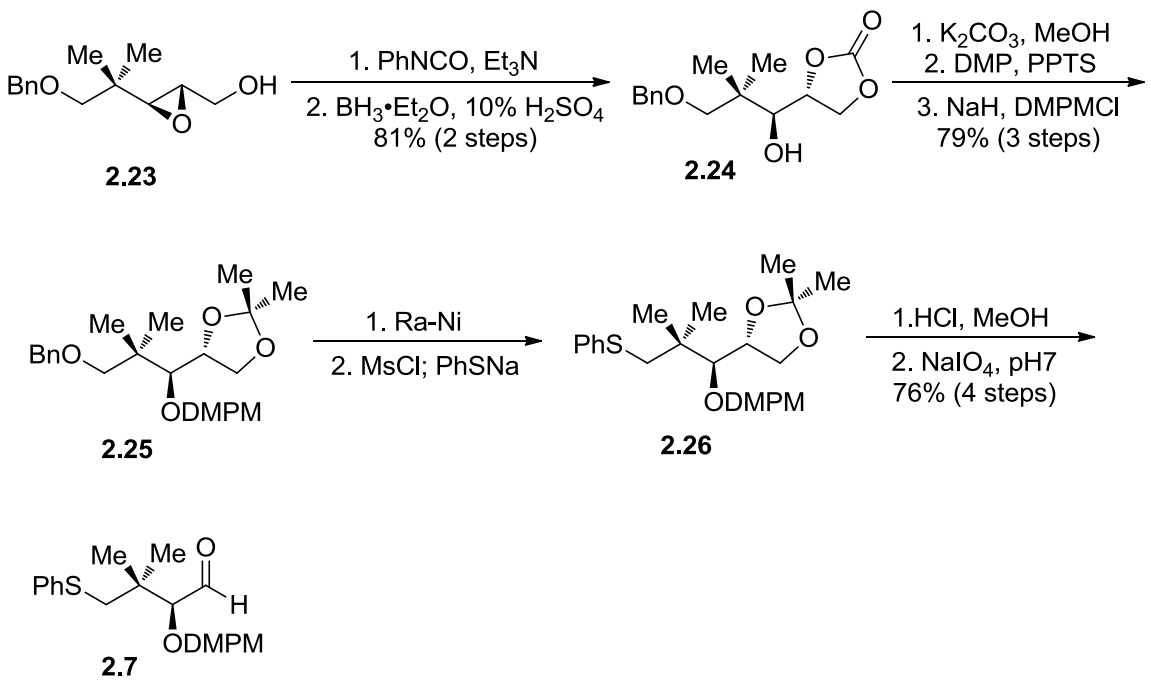


Figure 2.6. Synthesis of Aldehyde Subunit 2.7

The C_{26} and C_{27} stereocenters of the C-ring were bought from the commercially available L-threonine (Figure **2.7**). Conversion of the amine into an alcohol group was carried out by diazotization followed by its displacement with water. Fischer esterification of the carboxylic acid followed by acetonide formation provided the methyl ester **2.28** with an yield of 64% over three steps. Half reduction of the ester using DIBAL-H led to the formation of an aldehyde which was then one carbon homologated using Wittig reaction. The olefin was subjected to hydroboration-oxidation followed by the PCC oxidation providing the aldehyde **2.30**. The C_{24} stereocenter was installed using a substrate controlled addition of allenyl zinc to the aldehyde **2.7** giving a 8:1 mixture of diastereomers in favor of desired isomer. The newly formed alcohol was protected as a MPM ether and the methyl ester was installed using chloroformate. A conjugate addition of the stannane followed by the full reduction of the methyl ester gave alcohol. The free alcohol was protected as a BPS ether and the stannane was converted into an iodide by treating it with iodine. A substrate controlled addition of the lithiate of vinyl iodide **2.8** to the aldehyde **2.7** installed the C_{20} stereocenter providing 6:1 mixture of alcohols. Protection of the newly formed alcohol as TES group, deprotection of the DMPM group and oxidation of the resulting alcohol provided the ketone **3.24**. At this point, the phenyl sulfide was oxidized to a sulfone and the MPM group was removed using DDQ.The C-ring of the bryostatin was prepared as methyl ketal using TESOTf/TMSOMe. Thus the synthesis of C-ring was completed in 21 longest linear steps starting from L-threonine. The synthesis utilized commercially available amino acid in order to install two stereocenters. Remaining stereocenters were installed using chelation controlled addition reactions using the existing stereocenters.

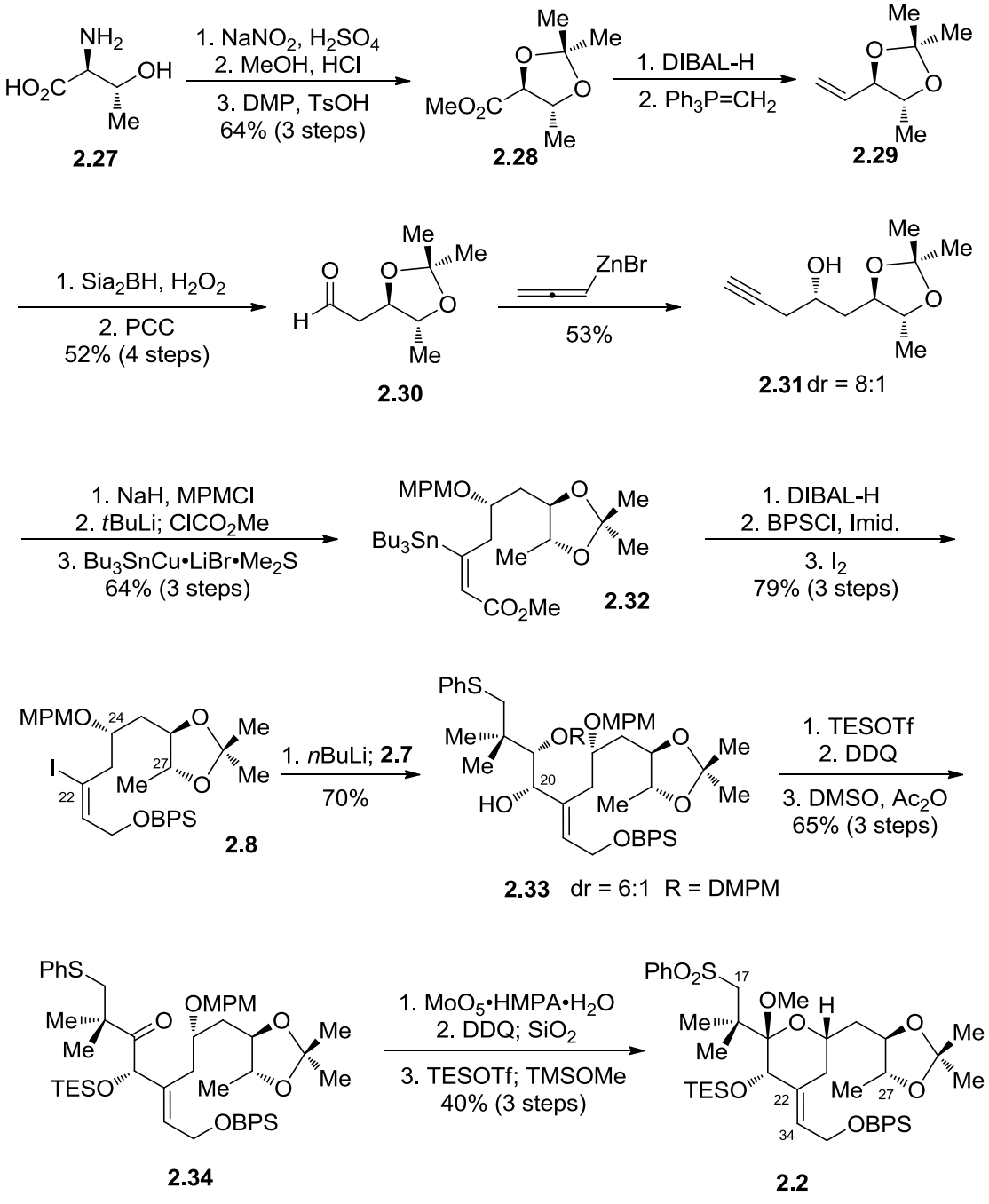


Figure 2.7. Completion of the C-ring

With two major fragments aldehyde **2.1** and sulfone **2.2** in hand, a Julia olefination using phenyllithium as a base provided the desired *E*-olefin in 60% yield after reductive elimination (Figure **2.8**). The choice of base for Julia olefination was crucial for the success of coupling reaction. Use of stronger base such as *n*-BuLi or *t*-BuLi led to the formation of byproducts whereas weaker bases like LDA or LEA resulted into incomplete deprotonation. In order to reveal the C_{20} alcohol, all the silicon protecting groups were removed using TBAF and selective protection of all alcohols as TBS ether except the C_{20} alcohol provided the desired product. Esterification of C_{20} alcohol as acetate followed by one more global silyl deprotection provided the triol **2.36**. Selective conversion of bis allylic alcohol to bismethyl ester installed the desired methyl esters on the B and the C-rings. The remaining C_3 alcohol was converted into the corresponding aldehyde. The C_3 stereocenter was then installed by using an asymmetric boron aldol reaction providing a 3:1 mixture of diastereomers in favour of the desired isomer. Removal of the acetonide led to the formation of a triol. A three step sequence involving TES protection of the triol, hydrolysis of the thioester and global silyl deprotection provided the trihydroxy carboxylic acid **2.40**. Although the macrolactonization of the thioester using thiophilic metal failed, use of Keck macrolactonization condition provided the desired C_{25} macrolactone in moderate yield. It is interesting to note that the acidic hydrolysis of the C_{19} methylketal could not be carried out in the presence of C_{20} acetate. It was thus necessary to remove C_{20} acetate for the acidic hydrolysis of C_{19} methyl ketal. The C_{26} alcohol of the triol was selectively protected as TBS ether and the remaining two alcohols were esterified as acetates. Removal of the TBS group completed the synthesis of bryostatin 7.

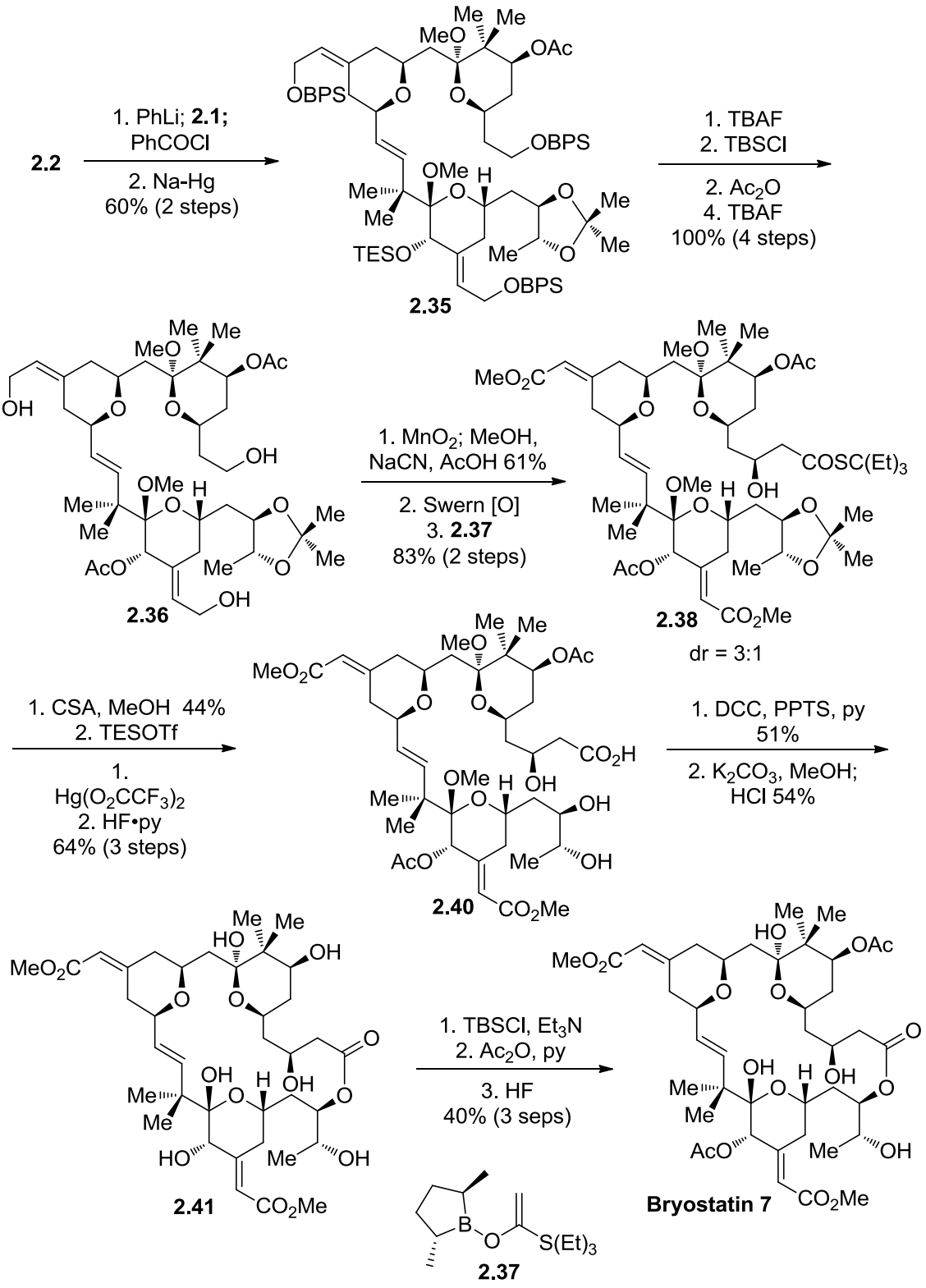


Figure 2.8. Masamune's Completion of Bryostatin 7

Thus the first total synthesis of bryostatin 7 by Masamune was accomplished in 42 longest linear steps and required more than 84 chemical steps. The synthesis provided half a milligram of synthetic bryostatin 7. Although Masamune targeted bryostatin 1 initially, setbacks in the route led to the synthesis of bryostatin 7. The major problem in the first generation route was removal of the C_3 MEM group. In order to remove such a problem in the future synthesis, the entire route had to be altered. The highlights of the synthesis involve the extensive use of Sharpless epoxidation, Masamune's chiral boron enolate as well as chelation controlled addition reactions to install the key stereocenters.

Evans' Total Synthesis of Bryostatin 2[18]

In 1999, Evans and coworkers disclosed the total synthesis of another member, bryostatin 2. The major disconnection used by Evans is similar to that of Masamune in that the B and C-rings would coupled by Julia olefination whereas the macrocycle would be formed by a Yamaguchi reaction (Figure **2.9**). The A and B-rings were joined by an alkylation of B-ring triflate with A-ring sulfone. This synthetic plan would divide the molecule to separate A, B and C-rings. It was initially envisioned to couple the fully functionalized three rings but due to the complication raised by functionalities mainly on the B and C-ring, it was decided to couple simpler fragments. Thus coupling was done using simplified B and the C-rings and were functionalized in late stage. The best order for the coupling of these rings was realized as B+C=BC+A=ABC although other sequences such as A+B=AB+C=ABC were attempted. The synthesis is heavily based on the methodologies developed in their labs such as Evans chiral auxiliary mediated aldol reaction as well as substrate controlled 1,3 antireduction of β-keto alcohols.

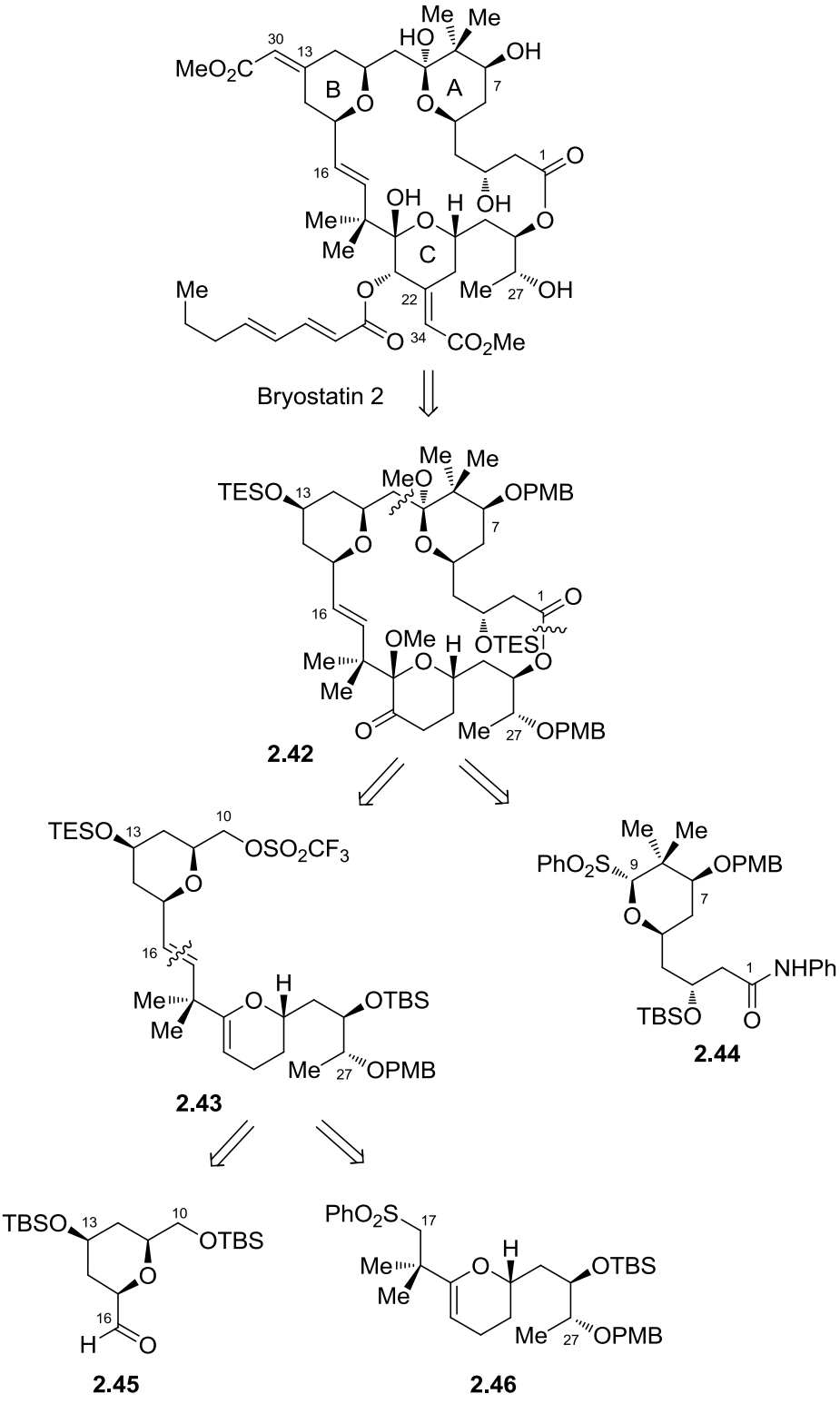


Figure 2.9. Evan's Retrosynthesis of Bryoststin 2

The synthesis of A-ring was started from aldehyde **2.47,** which was prepared in 5 steps from commercially available 2,2-dimethylpropane-1,3-diol (Figure **2.10**). A diastereoselective aldol reaction of aldehyde **2.47** using chiral chloroacetate oxazolidinone provided the chloroalcohol **2.48** in excellent diastereoseleectivity. A reductive removal of chlorine followed by removal of the chiral auxillary provided the diol which were differentiated by first converting it into a PMP acetal followed DIBAL-H reduction. The aldehyde was subjected substrate mediated 1,3-chelation controlled aldol reaction providing a four carbon homologated product in excellent yield and

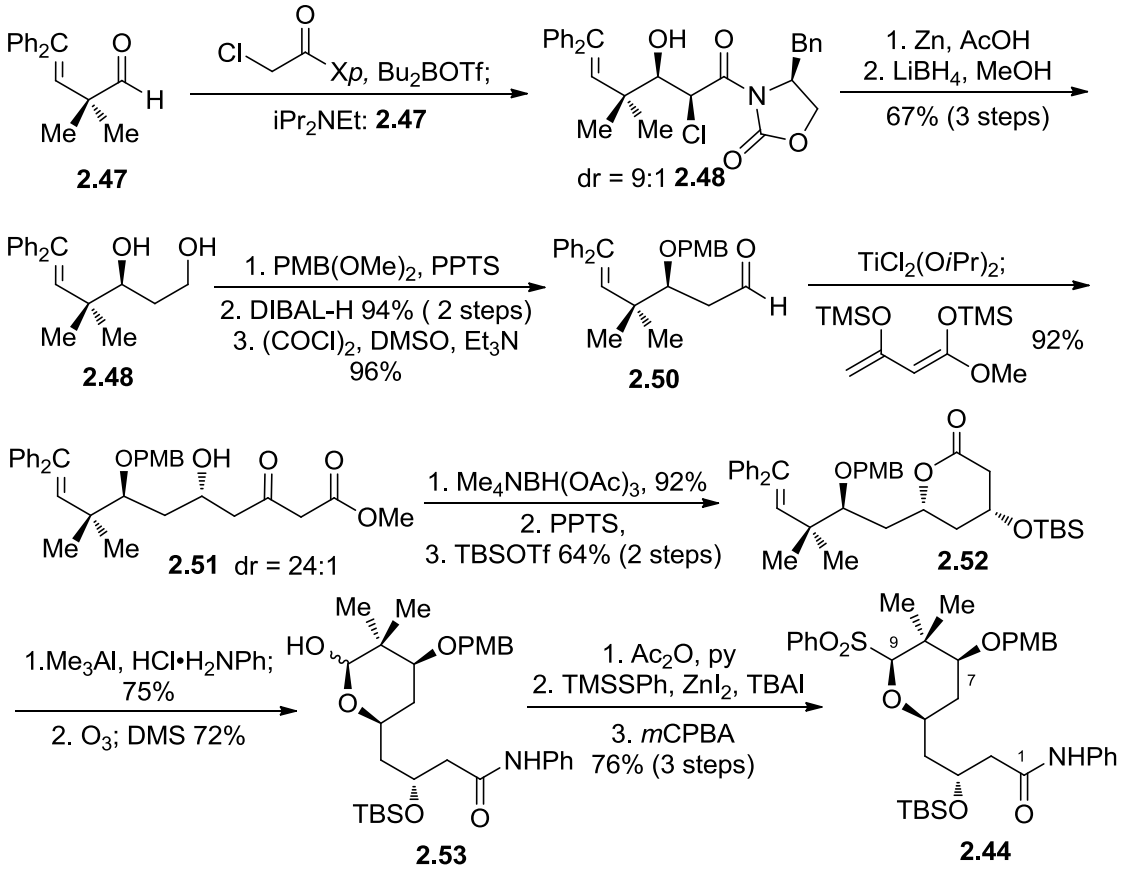


Figure 2.10. Evan's Synthesis of A-ring of Bryoststin 2

diastereoselectivity. One more substrate controlled antireduction of the beta keto alcohol provided the diol with high diastereoselectivity. The diols were differentiated by forming a six membered lactone with one of them and the remaining was protected as a TBS ether. Opening of the lactone to anilide followed by ozonolysis and subsequent cyclization provided the lactol as mixture of diastereomers which was carried on withoyt separation. Selective acetylation of the lactol and its displacement with phenyl sulfide provided the diastereometically pure sulfide which was oxidized using *m*-CPBA providing the A-ring sulfone in 20 steps from commercially available material.

Preparation of B the ring component also relied on the asymmetric aldol reaction and 1,3 anti reduction (Figure **2.11**). The B-ring was completed in 9 steps from aldehyde **2.54** which was in turn prepared in 2 steps from commercial (Z)-but-2-ene-1,4-diol via dibenzyl protection and ozonolysis. An asymmetric aldol reaction of the bis TMS enol ether using Evans' chiral py-box catalyst was used to install the C_{15} stereocenter. One more a 1,3 chelation controlled Saksena-Evans' reduction of the beta keto alcohol provided the diol in excellent diastereoselectivity. The diols were differentiated through lactone formation and TBS protection. Treatment of the lactone with benzyloxymethyl lithiate provided the lactol as single diastereomer which was reduced to pyran in high diasereoselectivity. The reduction also removed the PMB and TES protecting groups. Bis TBS protection followed by debenzylation and oxidation completed the synthesis of the B-ring of bryostatin.

The synthesis of the C-ring required the preparation of ketone **2.62** (Figure **2.12**). Subjection of the racemic alcohol **2.60** to Sharpless asymmetric kinetic resolution provided the enantiomerially pure alcohol which was protected as a PMB ether.

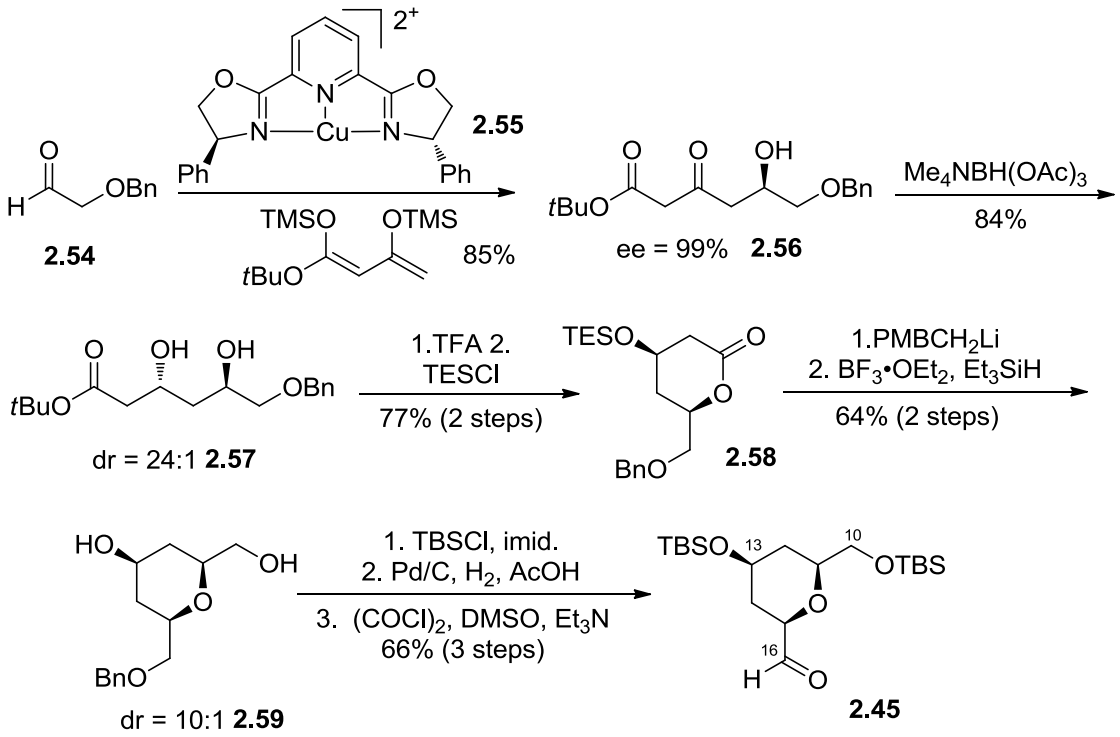


Figure 2.11. Synthesis of B-ring of Bryoststin 2

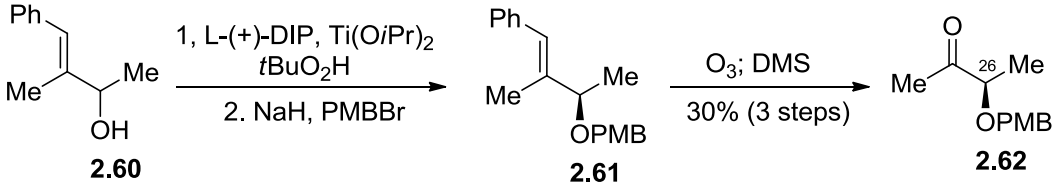


Figure 2.12. Synthesis of C_{24}-C_{27} Fragment

Ozonolysis of the olefin provided the ketone **2.62** in 30% yield over 3 steps.

Preparation of the aldehyde coupling partner for the aldol reaction is shown in Figure **2.13**. The precursor aldehyde **2.64** was prepared in 4 steps from the diol **2.63** via monotosylation, phenyl sulfide displacement, sulfide oxidation and alcohol oxidation. A Grignard addition to the aldehyde and oxidation of the resulting alcohol followed by oxidative cleavage of the terminal olefin provide the aldehyde **2.65**. Paterson borane aldol transformation provided the adduct **2.66** in high yield and diasteroselectivity. A directed Evans-Tishchenko reduction of the ketone not only installed the C_{23} stereocenter but also differentiated the diol after reduction. Protection of the newly formed alcohol as TBS ether followed by the hydrolysis of the PNB ester provided the keto alcohol **2.67** which

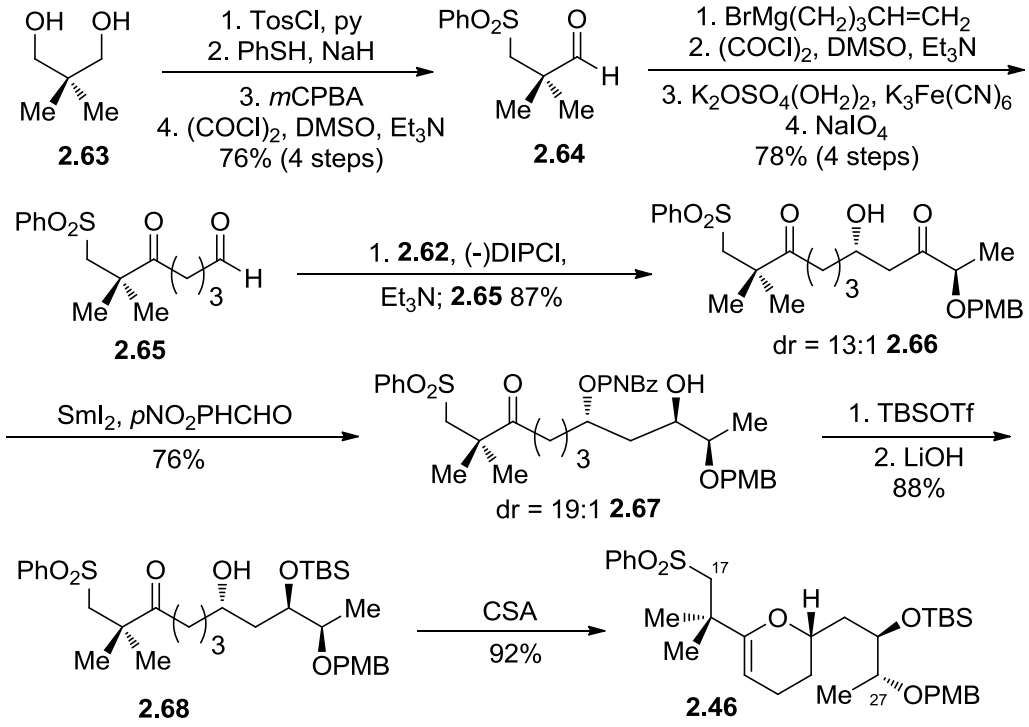


Figure 2.13. Synthesis of the C-ring

was converted to glycal **2.46** under dehydrative cyclization.

With the completion of the synthesis of all three rings, coupling of these fragments was undertaken (Figure **2.14**). The three rings could be coupled in different ways, either A+B=AB+C=ABC or B+C=BC+A=ABC. A more convergent coupling of the fully functionalized C-ring sulfone with B-ring aldehyde could not be carried out. It was eventually discovered that the later coupling sequence provided the better overall yield of the tricycle. Addition of the anion of the C-ring sulfone to the B-ring aldehyde **2.45** formed the hydroxyl sulfone which was in situ acetylated and finally eliminated providing the BC fragment. In order to combine it with the A-ring, the C_{10} alcohol was revealed by selective deprotection of the primary TBS group and converted into a unstable triflate which was immediately displaced with the dianion derived from the A-ring sulfone **2.44**. This reaction sequence provided the ABC tricycle in a 19 step longest linear sequence from commercially available starting materials. Attempts to carry this tricycle were frustrated due to the acid/base instability of the A-ring. Functionalization of tricycle core required the opening of the unstable A-ring lactol to the more robust TES protected ketone. The N-phenyl amide at C1 was switched for a benzyl ester. The acid sensitive C-ring glycal was converted into more stable methoxy ketone via epoxide formation, opening of the epoxide, equilibrium of the mehtylketal and oxidation of the alcohol. A global silyl deprotection using acidic methanol resulted in a triol with simultaneous formation of the A-ring methylketal. Selective protection of the C_3 and C_{13} alcohols followed by benzoyl deprotection provided the seco acid. Yamaguchi esterification to close the macrolactone was followed by the selective deprotection of the C_{13} TES ether to give alcohol **2.71**.

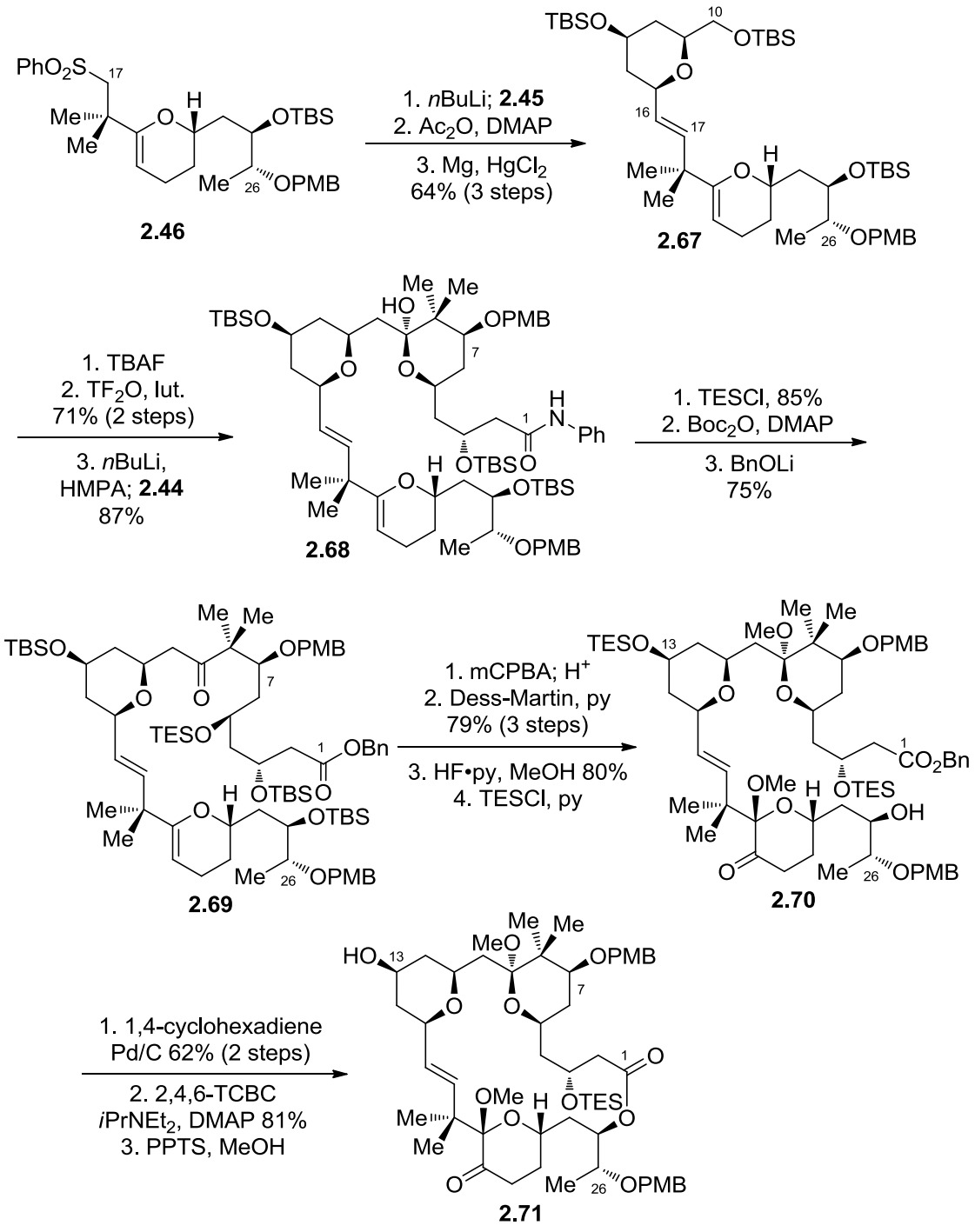


Figure 2.14. Coupling of the A-, B- and C-rings

With the macrocrolactone in hand, the functionalization of the B-ring was addressed first. The C_{13} alcohol was oxidized to a ketone using Dess-Martin oxidation (Figure **2.15**). An asymmetric HWE reaction of the bisketone with chiral *R*-BINOL phosphate proved to be regioselective and provided the desired *Z* olefin as a separable 6:1 mixture of isomers favouring the desired isomer. Functionalization of the C-ring involved an aldol reaction with methyl glyoxylate which the led to the formation of the aldol adduct. Dehydration of the adduct using Buress reagent provided the keto enoate in good yield as a 6:1 mixture of *E*:*Z* isomers. Initial attempts to reduce the ketone using Luche condition were unsuccessful due to low yield and poor selectivity. But use of CBS regent provided the desired alcohol in good yield and selectivity and was temporarily protected as methoxyacetate. Hydrolysis of the C_9 methylketal and TES deprotection was followed by saponification of the methoxyacetate. Further acidic hydrolysis removed the C_{19} methylketal and also equilibrated the C_9 hemiketal providing the tetraol **2.74**. A selective esterification of the C_{20} alcohol with octadienoic acid followed by global PMB deprotection completed the total synthesis of bryostatin 2.

Evans and coworkers thus completed the total synthesis of bryostatin 2 in 75 total steps and 42 longest linear steps. The synthesis provided 0.8 mg of the synthetic bryostatin 2. Evan's synthesis is based on an impressive use of methodology developed by Evans and coworkers earlier in their laboratory. Among all, the noteable methodologies are Lewis acid mediated substrate controlled 1,3-chelation controlled additions, chiral auxiliary mediated aldol reactions and a 1,3 directed reduction of the beta-keto alcohols using $Me_4BH(OAc)_3$ and SmI_2.

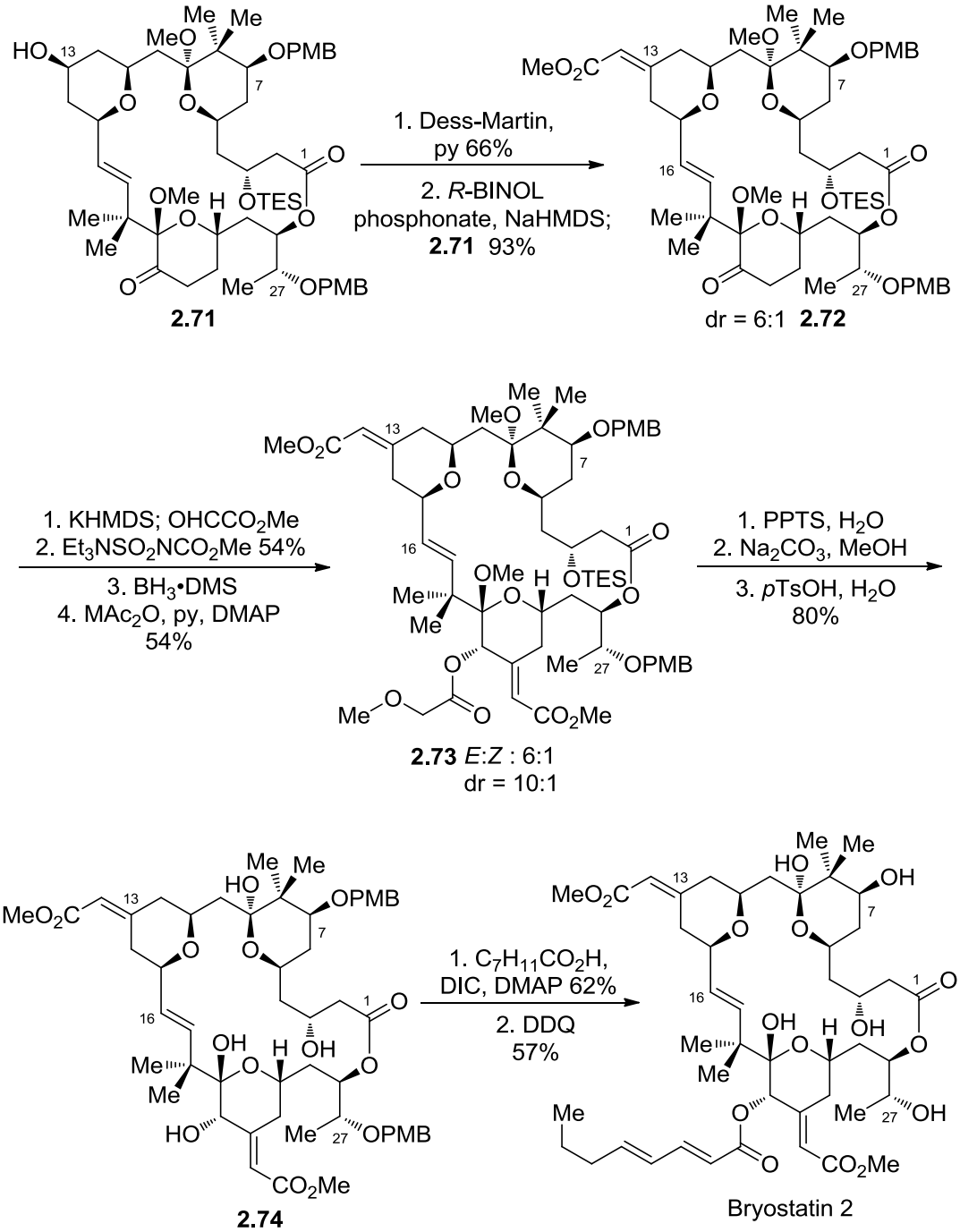


Figure 2.15. Completion of Bryostatin 2

Yamamura's Total Synthesis of Bryostatin 3[19]

Bryostatin 3, the most complex member of the family, was the third bryostatin to be synthesized by Yamamura and coworkers in 2000. Bryostatin 3 is more complex than other members of the family due to the presence of an unsaturated γ-ring ring on the C-ring. Similar to the previous two syntheses, this synthesis would be based on a convergent coupling of the C-ring system with the A-B-ring system via Julia olefination and macrolactonization (Figure **2.16**). The A and B-rings fragment were in turn were joined using dithane coupling of the acyclic A-ring anion with B-ring iodide. The A and C-rings would be prepared by acid catalyzed ketalization of the corresponding acyclic keto alcohols whereas B-ring was formed using an asymmetric hetero Diels-Alder reaction. This would require the preparation of the four fragments.

Starting from D-mannitol, the preparation of B-ring required 11 steps (Figure **2.17**). Bisacetonide formation of the terminal alcohols followed by oxidative cleavage of the middle diol provided the chiral aldehyde **2.84.** The aldehyde was subjected to a herero Diels-Alder reaction using Danishefsky diene **2.85**. This substrate controlled reaction provided the dihydropyranone **2.86** as a single isomer. A vinyl cuprate conjugate addition on the resulting dihydropyranone provided the *trans*-pyran. The ketone group was protected as methyl acetal which was followed by ozonolysis of the terminal olefin providong a aldehyde. The *trans* pyran was then converted into *cis* by epimerization of the C_{15} stereocenter with potassium carbonate. Reduction of the aldehyde and benzyl protection was followed by removal of the acetonide and cleavage of the diol to an aldehyde. Two more steps involving an aldehyde reduction and iodination completed the synthesis of the B-ring.

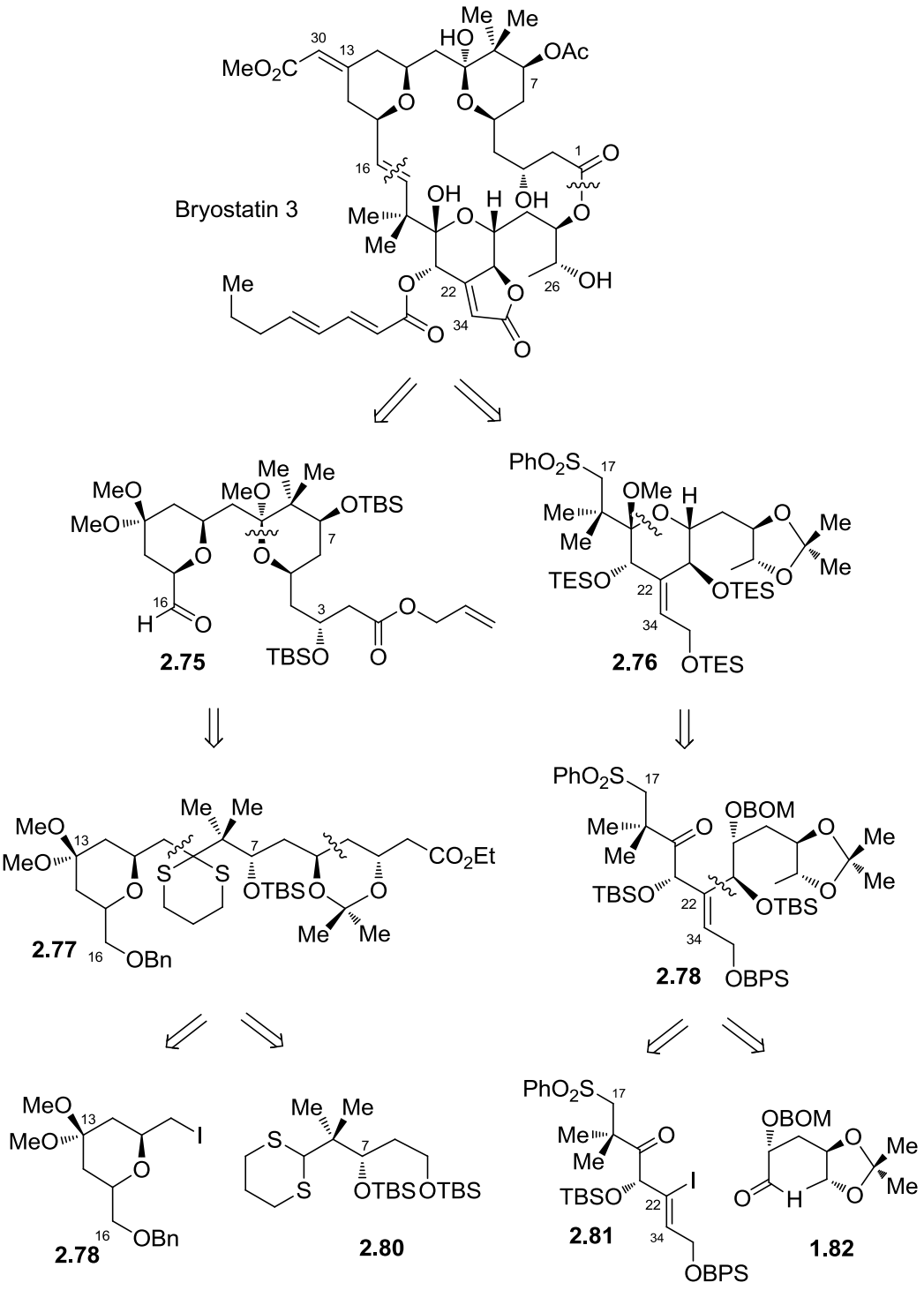


Figure 2.16. Yamamuran's Retrosynthesis of Bryostatin 3

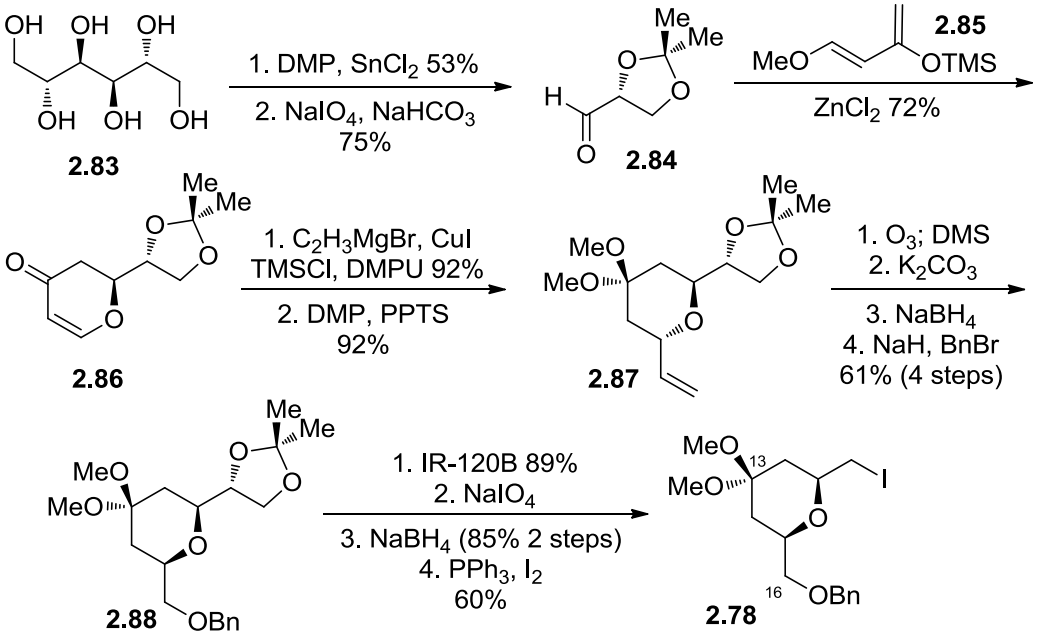


Figure 2.17. Preparation of C_{10}-C_{16} Fragment

The synthesis of the A-ring commenced from the commercially available diol **2.89** which after five steps provided the epoxy alcohol **2.91** (Figure **2.18**). Regioselective opening of the epoxide, bis-TBS protection and benzyl deprotection provided the alcohol which was oxidized and converted into dithaine **2.93**. Displacement of the B-ring iodide with the anion derived from the dithaine led the coupling of A and B-ring fragment. Selective removal of the primary TBS group followed by oxidation provided the aldehyde **2.94** for an asymmetric aldol reaction. Deprotonation of the menthone enol ether **2.95** followed by addition to aldehyde **2.94** provided the desired alcohol in excellent selectivity. Removal of the chiral auxillary followed by the 1,3 chelation controlled Evans-Saksena reduction established the C_3 stereocenter. A mercury mediated

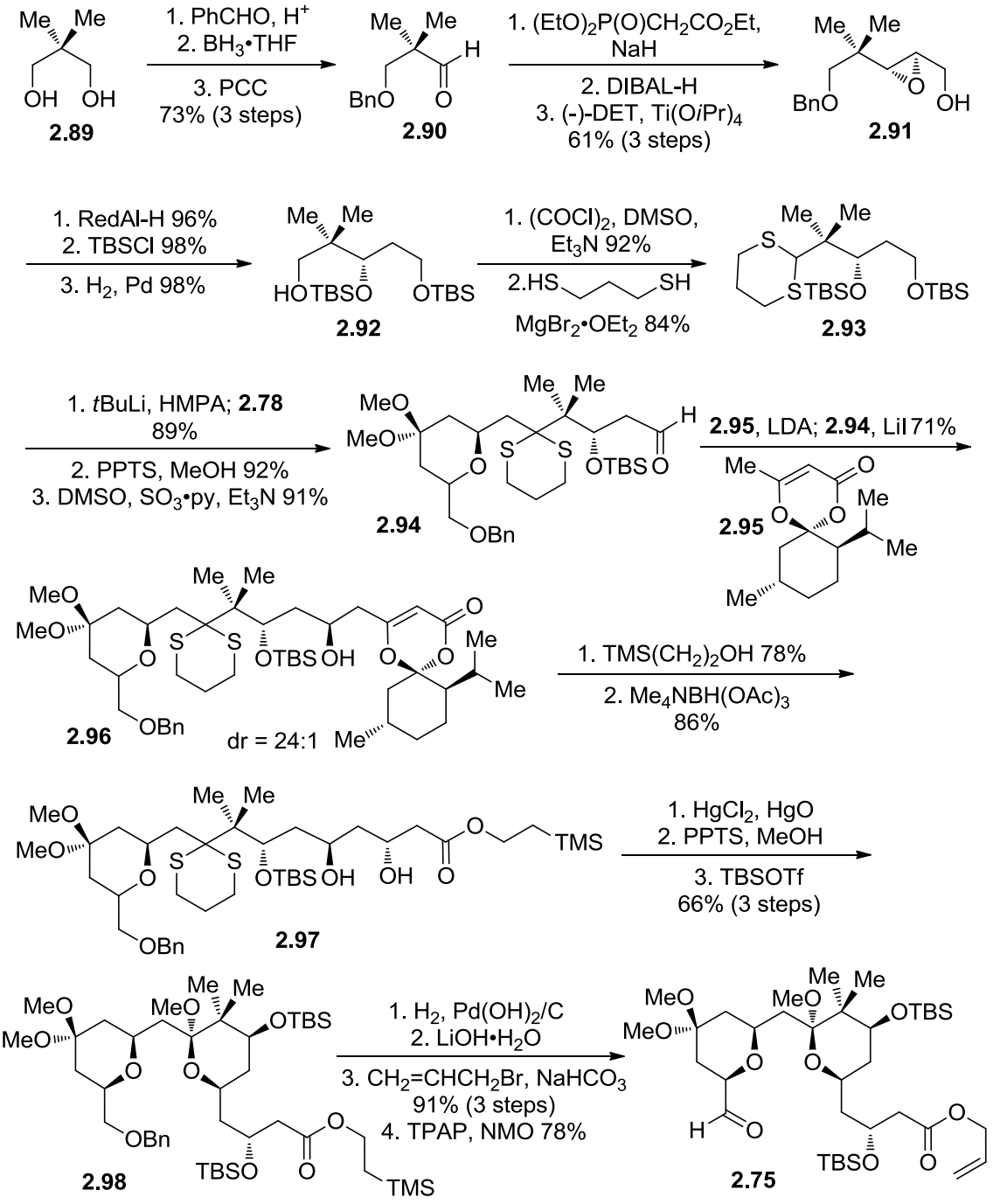


Figure 2.18. Completion of the A-B-ring

dedithianolization followed by acid catalyzed methyl ketalization secured the A-ring. Debenzylation, basic hydrolysis of the ester, alkylation of the acid followed by the oxidation of the alcohol completed the synthesis of the A-B-ring system of bryostatin 3.

The aldehdye precursor **2.82** for the synthesis of C-ring was prepared from D-glucose (Figure **2.19**). Bisacetonide formation, tosylation of the remaining alcohol, elimination and hydrogenation provided the tetrahydrofuran **2.100** with correct stereochemistry. The more labile acetonide was selectively removed and the terminal alcohol was tosylated. Reduction of the tosyl group installed the C_{27} methyl group. Removal of the remaining acetonide was accompained by opening of the lactol and its subsequent conversion into a dithaine. The triol was once again subjected to regioselective terminal acetonide formation and BOM protection. Removal of the dithaine provided the C_{22}-C_{27} aldehdye **2.82**.

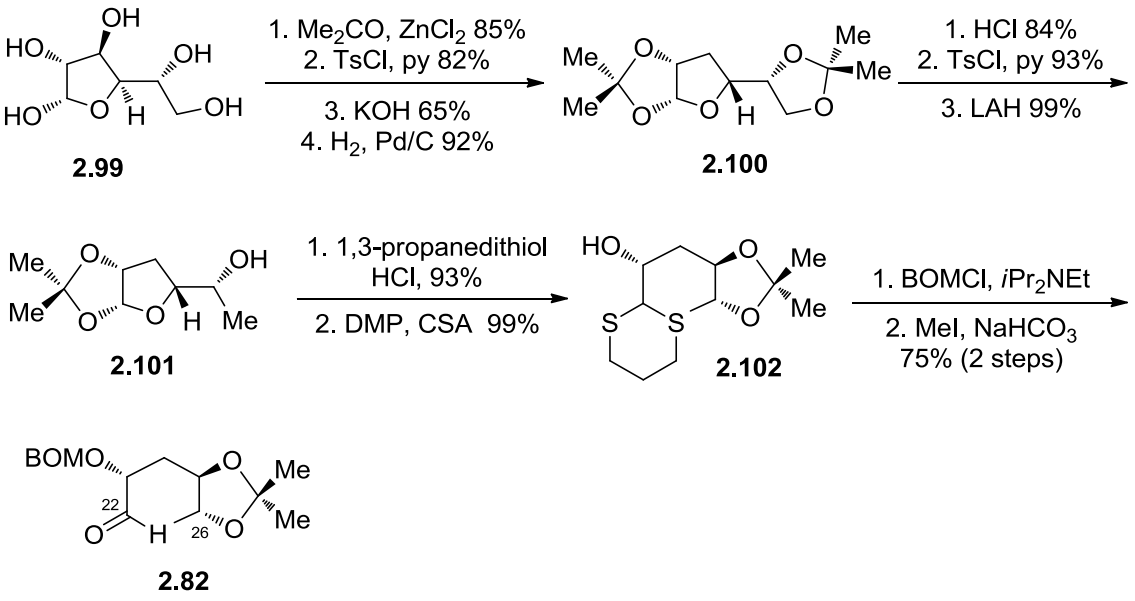


Figure 2.19. Yamamura's Synthesis of C-ring

Similar to the A-ring synthesis, the synthesis of C-ring also commenced from diol **2.89** (Figure **2.20**). A six step protocol furnished the olefin **2.104** which was subjected to SAD followed by acetonide protection. Half reduction of the ester provided the aldehyhde which was then converted into a dibromoalkene with one carbon homologation. Conversion of the dibromo species to a propargylic alcohol was followed by vinyl iodide formation. The acetonide protecting group was switched for a PMP acetal

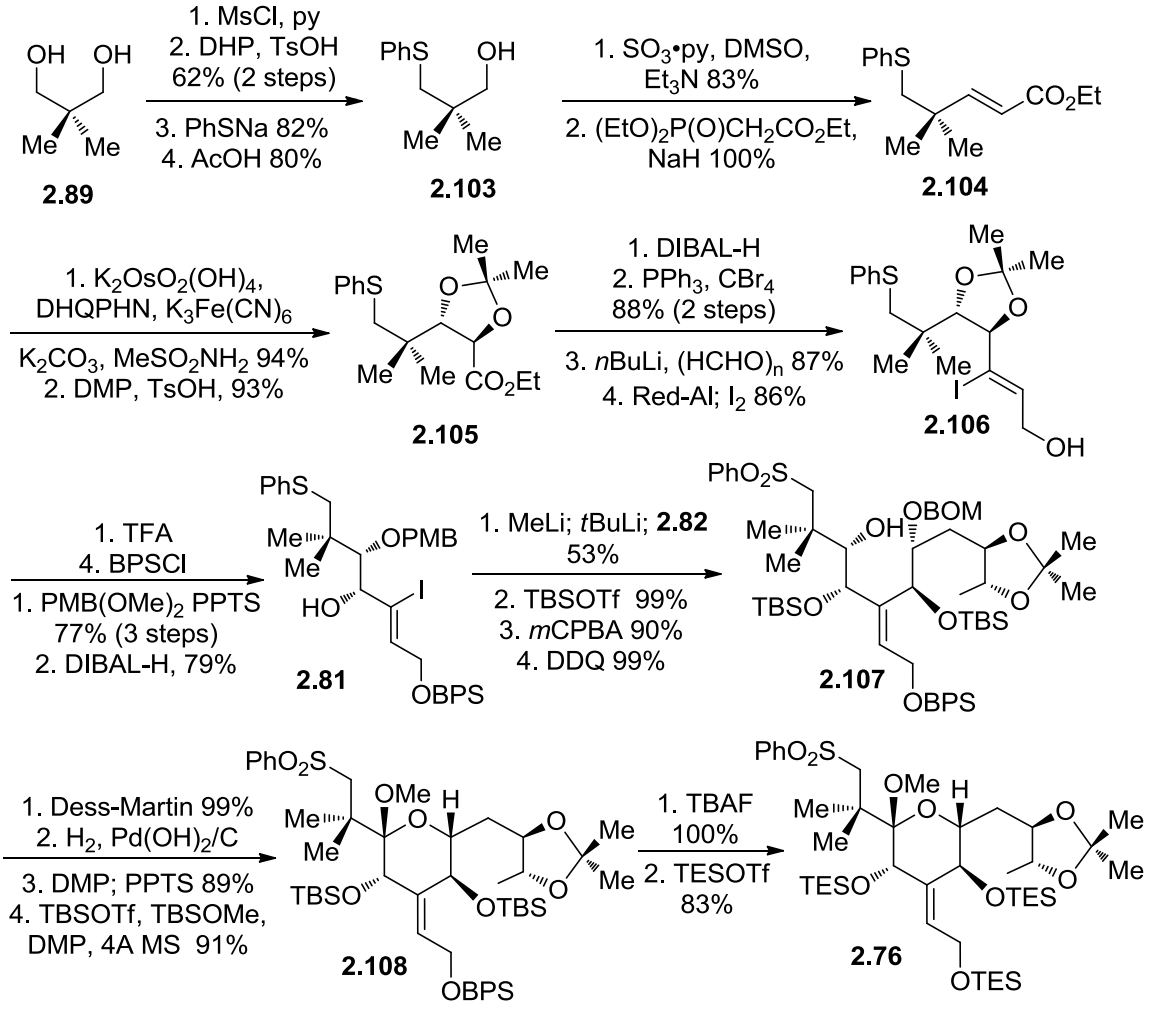


Figure 2.20. Synthesis of C-ring Sulfone

and regioselective opening of which provided the alcohol **2.81**. With both iodide **2.81** and aldehyde **2.82** in hand, the coupling of these fragments was attempted using Nozaki-Himaya-Kishi reaction which proved to be unsuccessful. A sequential treatment of the iodide **2.81** with MeLi and *t*-BuLi followed by addition of the aldehyde provided the desired product as 3:1 mixture of diastereomers. It is interesting to note that Masamune also performed similar coupling with switched iodide and aldehyde pieces and was able to get higher yield and selectivity (*vide supra*). Protection of the alcohol, oxidation of the sulfone and removal of PMB group provided the alcohol which was oxidized to a ketone. The BOM group was removed and keto alcohol was cyclized to hemiketal which was finally converted into methylketal. A global exchange of the silyl groups with TES group completed the synthesis of C-ring.

The key Julia coupling of the A-B-ring aldehyde with the C-ring sulfone was accomplished providing the tricycle in moderate yield (Figure **2.21**). Selective deprotection of the C_{21} and C_{22} alcohols followed by Lay oxidation afforded the α,β-unsaturated γ-lactone. One more site selective silyl deprotection provided the C_{20} alcohol which was esterified with the bryostatin side chain using Yamaguchi reaction. Selective removal of the acetonide and the C_3 TBS ether provided a triol in which C_3 and C_{27} alcohols were selectively protected as TES ethers leaving the C_{26} alcohol free. The seco acid was prepared after the removal of the allyl ester and was subjected to Yamaguchi macrolactonization.

With the macrolactone in hand, the functionalization of the B-ring commenced with removal of the methylacetal on the B-ring. An asymmetric HWE reaction of the ketone with the anion of BINOL phosphonate installed the α,β-unsaturared ester with

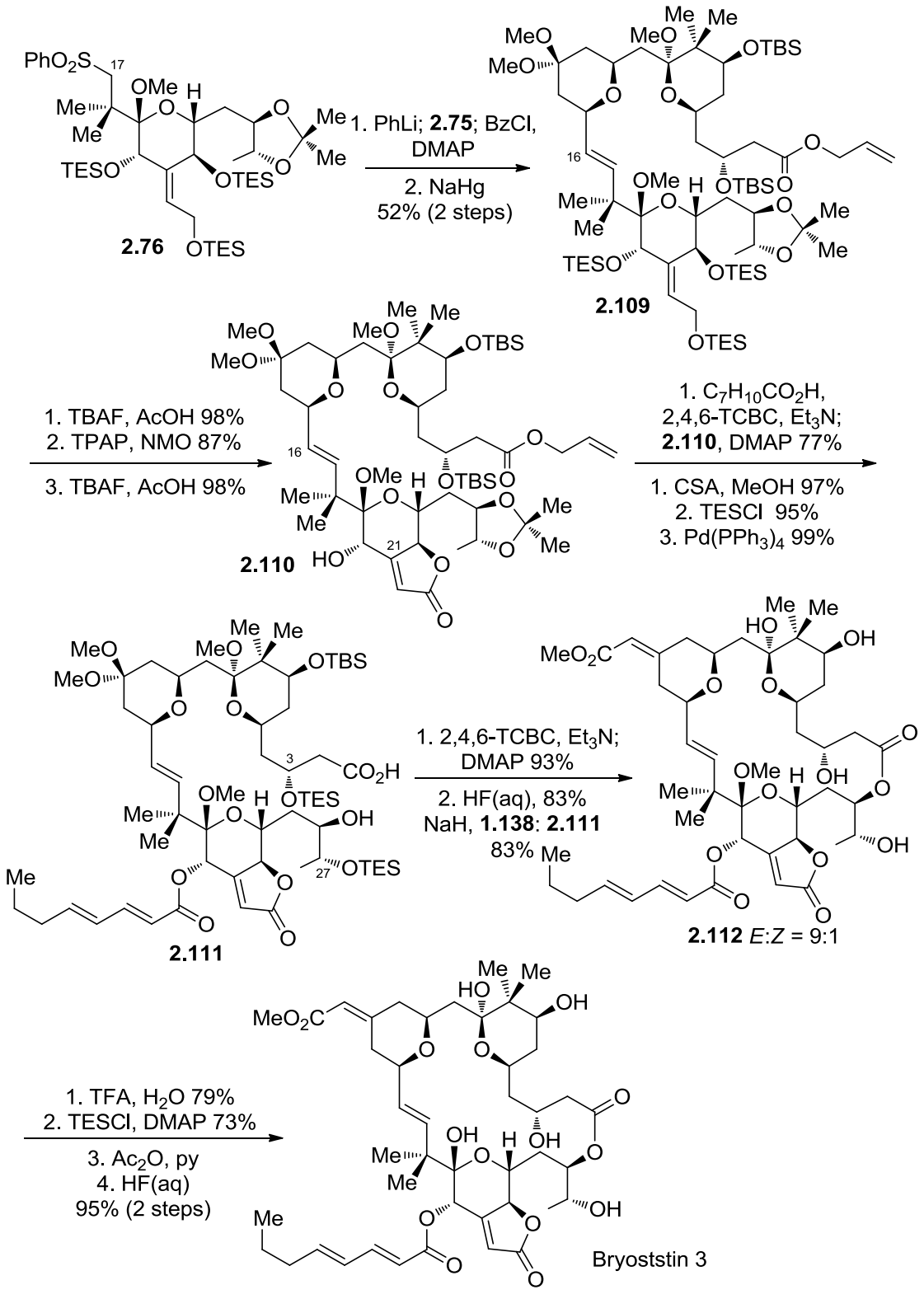


Figure 2.21. Yamamura's Completion of Bryostatin 3

good selectivity. It is interesting to note that the HWE reaction was less selective in Evan's synthesis of bryostatin 2. Hydrolysis of the C_{19} methylketal was found to be problematic initially but was finally accomplished using TFA/H_2O. A temporary protection of C_{26} alcohol followed by acetylation of C_7 alcohol and final deprotection completed the synthesis of bryostain 3.

In summary, Yamamura completed the synthesis of most complex member of bryostatin in 43 steps (longest linear sequence) and 87 steps total. This synthesis utilized carbohydrates as a major source of chiral pool. Although Yamamura and coworkers said that they made 25 mg of bryostatin 3, the biological evaluation on this material is yet to be reported.

Trost's Total Synthesis of Bryostatin 16[19]

Trost and coworkers have recently published the total synthesis of bryostatin 16. Structurally, bryostatin 16 is one of the less complex bryostatins due the absence of oxidatin at C_{19}-C_{20} position on the C-ring. In addition, bryostatin 16 has significantly lower binding affinity (Ki = 116 nM) compared to other bryostatins (bryostatin 1, for example, has binding affinity of 1.4 nM). However, with an aim to elaborate to other bryostatins, Trost and coworkers targeted the synthesis of bryostatin 16. The synthetic plan is different from the previous three syntheses in that the C-ring is formed by an alkyne-alkyne coupling with concurrent formation of the macrocycle (Figure **2.22**). The B-ring was formed by a palladium catalyzed alkene-alkyne coupling followed by cyclization. The A-ring was formed by the acid catalyzed methyl-ketalization of the corresponding keto alcohol.

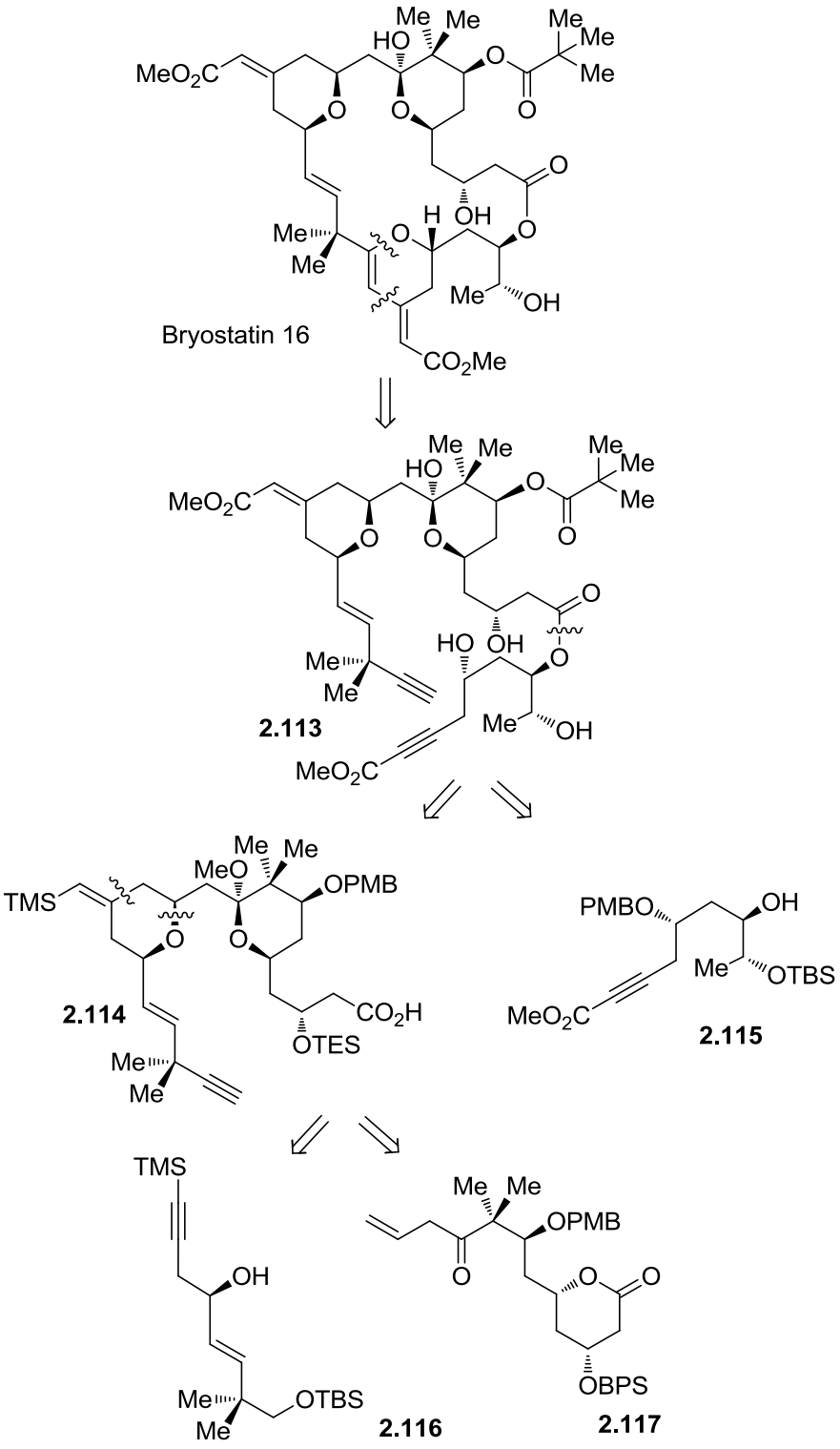


Figure 2.22. Trost's Retrosynthesis of Bryostatin 16

The synthesis began with the preparation of the alkene fragment **2.117** (Figure **2.23**). Starting from aldehyde **2.118** (prepared in two steps from commercially available diol), a brown allylation followed by PMB protection installed the C_7 stereocenter. Oxidative cleavage of the diol derived from the olefin provided the aldehyde which was subjected to a 1,3 chelation controlled Mukiyama aldol addition using TMS enol ether as the nucleophile. One more hydroxy directed substrate controlled reduction installed the C_3 stereocenter. The diols were differentiated by a selective formation of six membered lactone using Otera's catalyst. Removal of the TBS ether, oxidation to aldehyde followed by allylindium addition and oxidation provided the olefin fragment **2.117**.

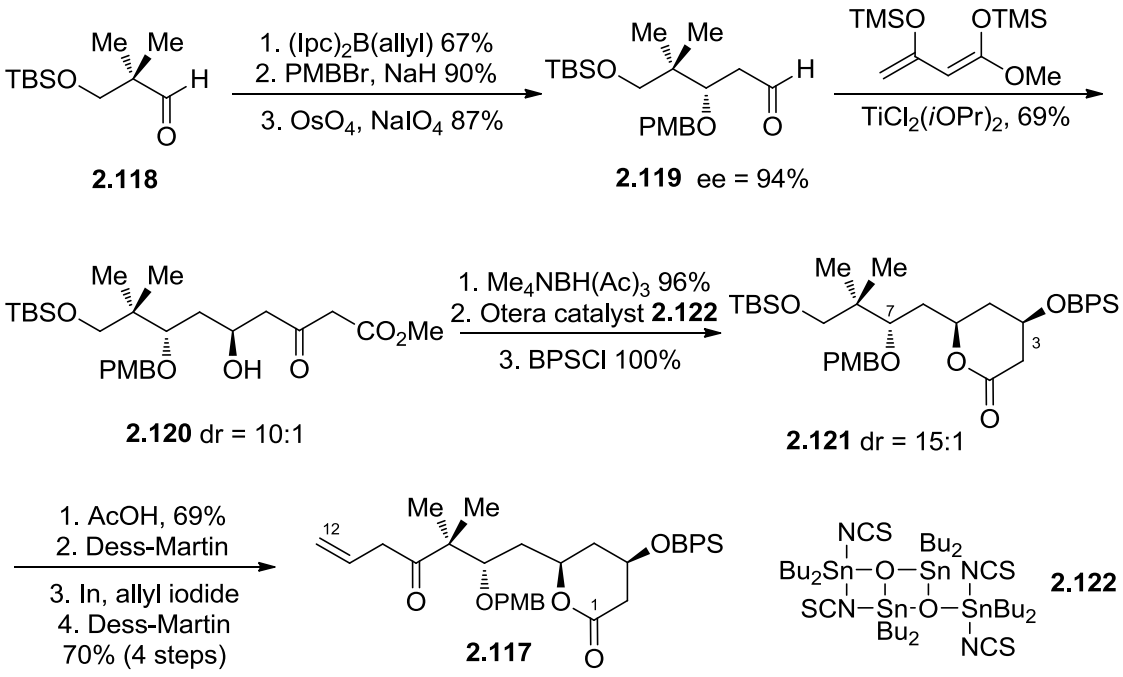


Figure 2.23. Synthesis of C_1-C_{12} Fragment

The alkyne partner **2.116** for the alkene-alkyne coupling was also prepared from the aldehyde **2.118** (Figure **2.24**). A two carbon homologation followed by indium mediated propargylation provided the racemic alcohol which was oxidized and reduced bromide followed by oxidation provided the aldehyde **2.125**. The aldehyde was homologated to an alkyne using Ohira-Bestmann reaction. Removal of the BPS ether with CBS reagent. With both partners in hand, a ruthenium catalyzed cascade alkene-alkyne coupling /conjugate addition generated the B-ring in moderate yield. Bromination of the vinyl silane was followed by transesterification/methykletal formation with CSA

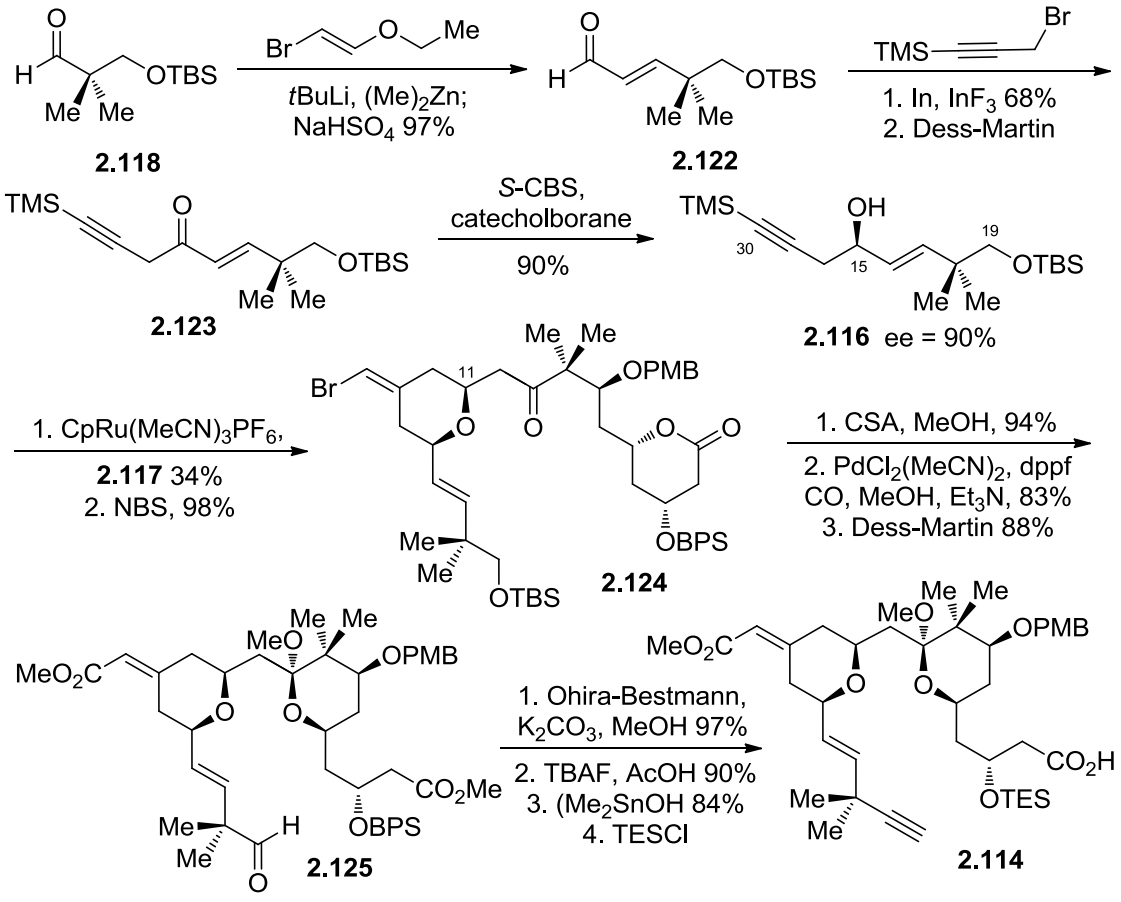


Figure 2.24. Completion of Northern Hemisphere

in MeOH which also deprotected the TBS group. A palladium mediated carbonylation of the B-ring followed by hydroxyl directed saponification and reprotection of the alcohol completed the synthesis of the northern hemisphere.

The alkyne fragment **2.115** was prepared from D-glactonic acid 1,4 lactone **2.126** (Figure **2.25**). All three stereocenters in this piece were bought from the starting material. Conversion of the primary alcohol to a bromide followed by acetylation provided the triacetate. The bromide was reduced to a methyl group and hydride reduction provided the tetraol which was protected as bisacetonide. Selective removal of the more labile primary acetonide followed tosylate formation which under basic conditions yielded the epoxide **2.128**. Opening of the epoxide and protecting group manipulation furnished the alcohol **2.115** in ten steps from commercially available material.

The coupling of the alcohol **2.115** and acid **2.114** under Yamaguchi condition provided the entire carbon framework of bryostatin 16. A bis-PMB deprotection led to the formation of diol ready for the critical alkyne-alkyne coupling. The optimized condition using 12 mole% $Pd(OAc)_2$ and 15 mol% tris(2,6-dimethoxyphenyl) phosphate ligand provided the macrocycle in moderate yield. Formation of the C-ring using a gold catalyzed 6-*endo*-*dig* cyclization closed the C-ring. Conversion of the C_7 alcohol into its pivalate followed by global deprotection of the silyl groups completed the synthesis of bryostatin 16.

Trost's synthesis of bryostatin 16 took 28 longest linear steps (41 chemical steps) from commercially available 2,2-dimethylpropane-1,3-diol. Their synthesis provided 0.4 mg of bryostatin 16. The synthesis utilizes their atom economical alkene-alkyne and alkyne-alkyne coupling to construct the B and C-rings of bryostatin 16.

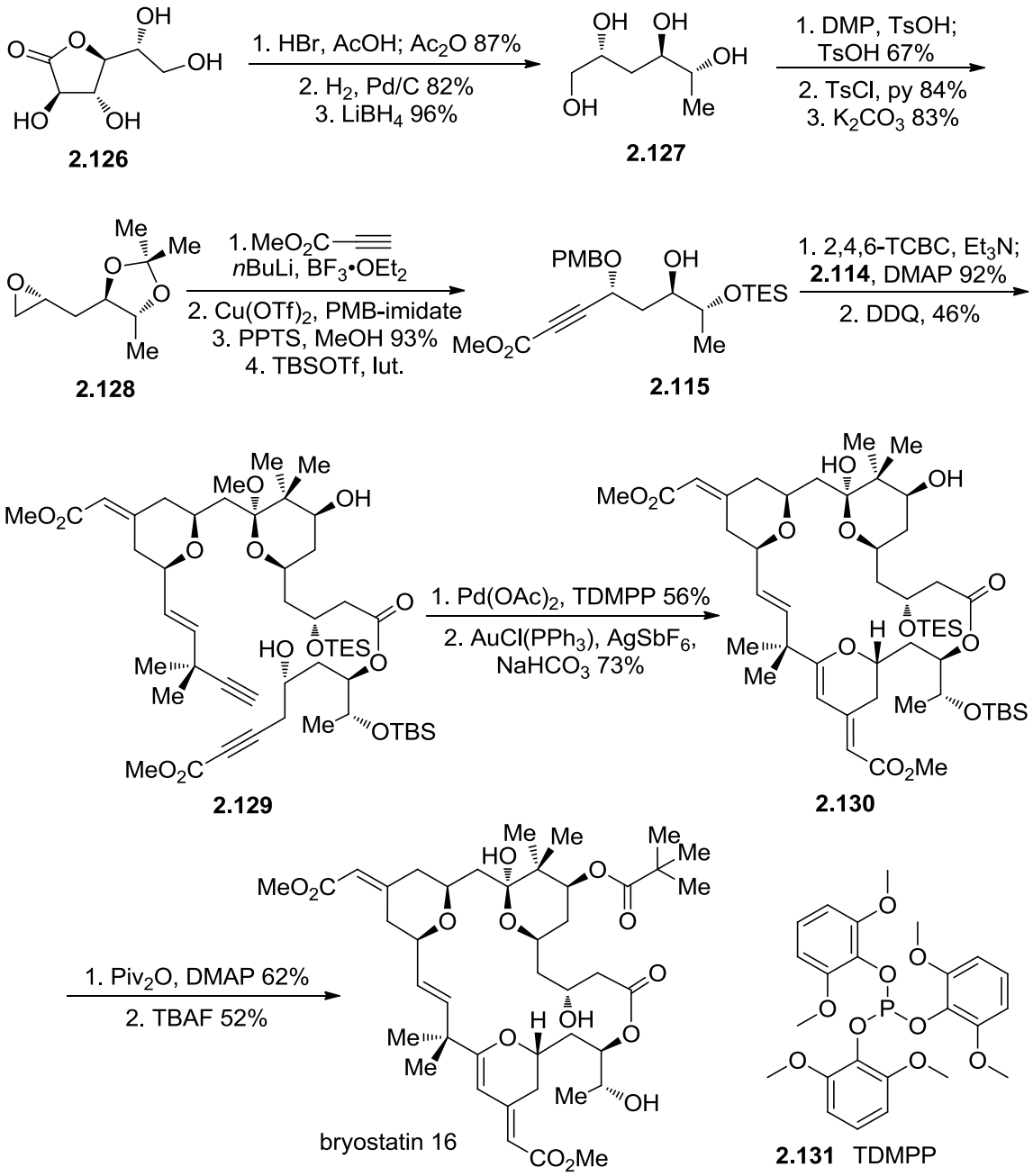


Figure 2.25. Trost's Synthesis of Bryostatin 16

With the completion of bryostatin 16, Trost and coworkers attempted to elaborate the intermediate **2.131**, which was prepared en route to bryostatin 16, to other bryostatins (Figure **2.26**).[24] Unfortunately, the dihydroxylation of the C_{19}-C_{20} glycal using Sharpless asymmetric AD-mix catalyst was unsuccessful. As an alternative, oxidation of the C_{19}-C_{20} olefin using rhenium oxide provided the undesired epoxide **2.132**. Opening of the epoxide followed by acetylation of the resulting alcohol and global deprotection provided 20-*epi*-bryostatin 7. Thus the conversion of bryostatin 16 like intermediates to other bryostatins was unsuccessful.

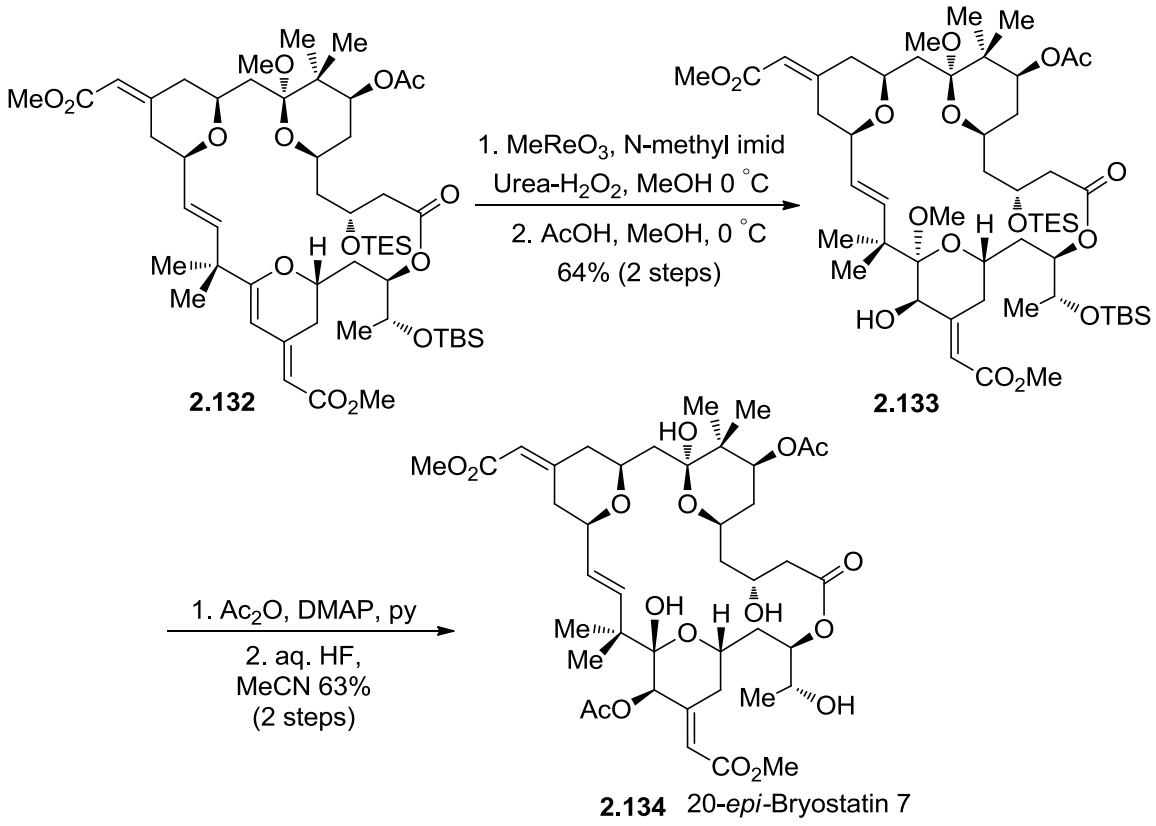


Figure 2.26. Attempted Elaboration of 2.132 to Bryostatin 7

Results and Discussion

The extremely low availability of the bryostatin 1 from natural as well as other sources has affected the further clinical development of this promising therapeutic lead. Moreover, synthesis of bryostatin 1, the most clinically used member of the family, has either not been attempted or not been successful so far. This led our group to direct studies towards the total synthesis of bryostatin 1.

Towards this end, our group has developed a powerful methodology for the construction of the *2,6*-disubstituted-*4*-methylene tetrahydropyran, a structural motif present in the B-ring of all the bryostatins.[25] Also referred as "pyran annulations," the process involves the coupling of a β-hydroxyallyl trimethylsilane **2.137** to an aldehyde **2.138** in the presence of TMSOTf at -78°C providing the *cis*-pyran **2.140** as a single diastereomer in high yield (Figure **2.27**). The reaction involve the formation of one carbon-carbon bond and one carbon-oxygen bond, one additional stereocenter, a six

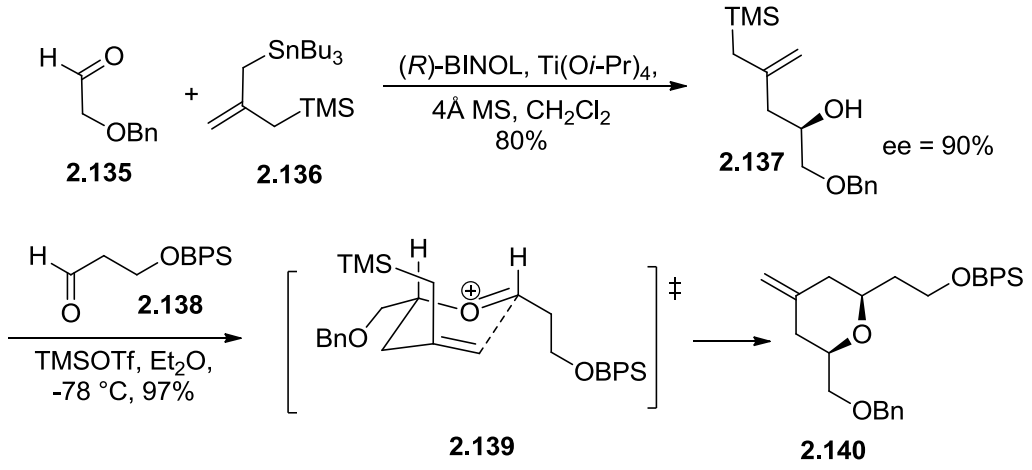


Figure 2.27. Pyran Annulation Methodology

membered ring and an exomethylene handle. The starting material β-hydroxyallyl trimetylsilane **2.137** can be prepared in high yield and enantiomeric excess by a catalytic asymmetric allylation (CAA) of corresponding aldehyde **2.135**.

The pyran annulation methodology allows for the synthesis of the pyran building blocks like **2.140** in just three steps from commercially available starting material. Using this methodology, our group has prepared a number of bryostatin analogues in which the B-ring or both A and B-rings of the bryostatins have been replaced by simple pyrans (Figure **2.28**). In Merle 23, both A and B-rings of bryostatin 1 have been replaced by exomethylene pyrans.[26] On the other hand, in the Merle 27, the exomethylene in the A-ring pyran has been cleaved to a ketone, reduced and acetylated.[27] Merle 28 and Merle 30 are our most bryostatin 1 like analogues with difference being just at one point. In Merle 28,[28] the B-ring is replaced by a simple pyran whereas in Merle 30,[29] the α,β-unsaturated methyl ester has been prepared by a Horner-Wadsworth–Emmons olefination of the ketone derived from the cleavage of the exocyclic olefin. In addition to our group, Wender's group has also used an intramolecular version of the pyran annulation in the construction of bryopyran analogues **2.141** and **2.142**.[30]

Retrosynthetic Analysis of Bryostatin 1

As mentioned earlier, three of the four previous total syntheses use the same major disconnection. Masanune, Evans and Yamamura construct the C_{16}-C_{17} olefin by using Julia coupling whereas a macrolactone ester is formed by a intramolecular lactonization fragment and a southern C- ring fragment. Trost's strategy is different from the previous three in the sense that C-ring is formed by a late stage intramolecular alkene-

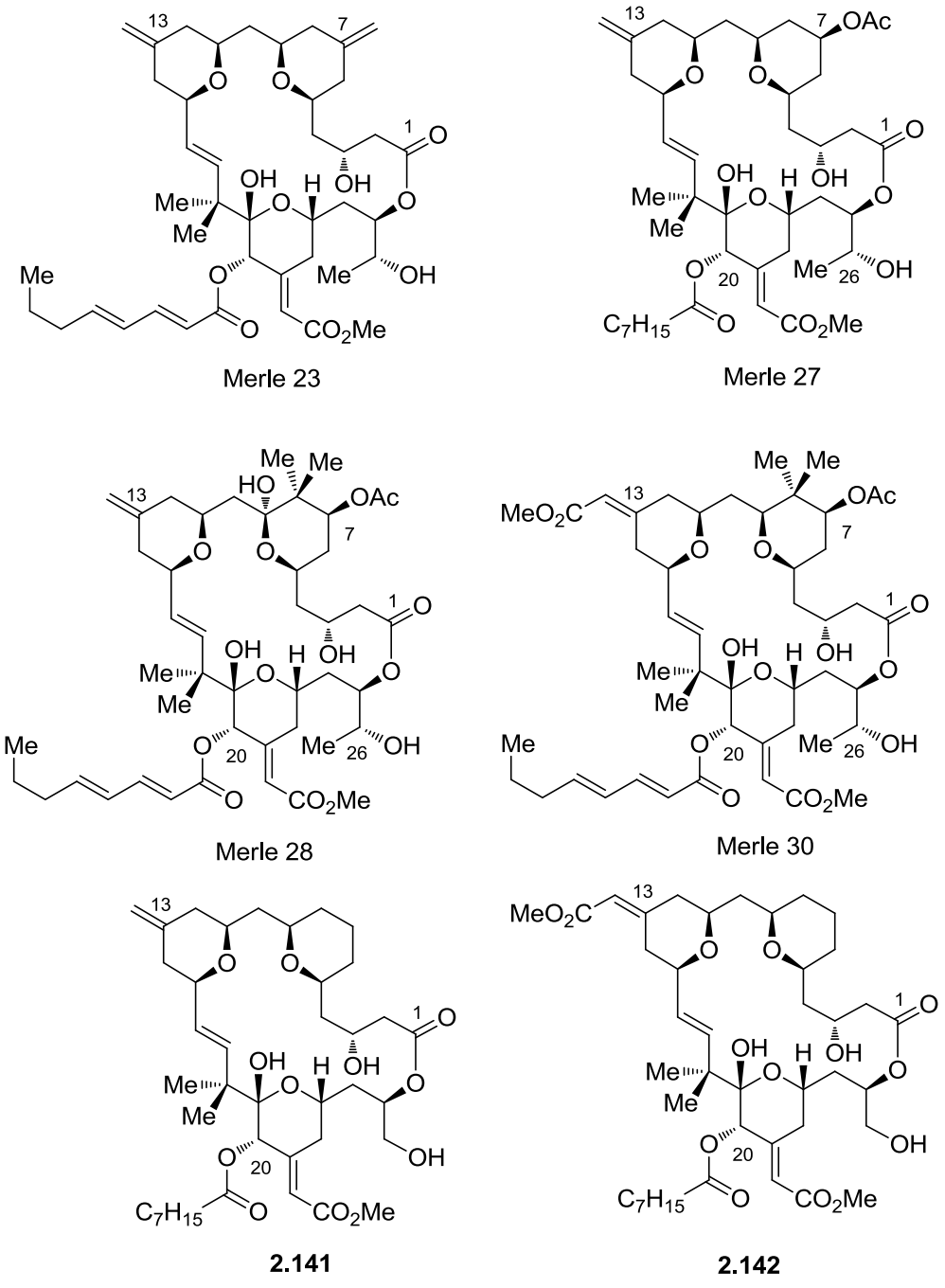


Figure 2.28. Representative Analogues Prepared Using Pyran Annulation

alkyne coupling followed by conjugate addition. Instead of conventional disconnections that divide the molecules into northern and southern parts, we sought a novel approach for bryostatin 1 synthesis. The discovery of the novel pyran annulation reaction and its successful application in the total synthesis of various bryostatin analogues led us to pursue this remarkable process in the total synthesis of the bryostatin 1 itself.

Our first generation retrosynthesis of bryostatin 1 is shown in Figure **2.29**. This approach utilizes a late stage functionalization of the B and C-rings to form a tricyclic macrolactone **2.143**. The B-ring of the tricycle was envisioned to be constructed from a pyran annulation between the A-ring aldehyde **2.145** and C-ring silane **2.144**. This synthetic plan was utilized in the synthesis of various bryostatin analogues.[28,29] Although this plan was successful in the synthesis of bryopyran analogues, the late stage functionalization of the C-ring in tricyclic compound **2.143** posed a number of problems. Most notable of these is the aldol condensation of the ketone **2.143** with methylglyoxylate in order to install the C_{22}-C_{34} α,β-unsaturated ester. This reaction was problematic due to the simultaneous aldol reaction on the C_7 acetate. The other problem is the low selectivity during the reductiuon of the C_{20} ketone in the advanced substrates. Moreover, this synthetic plan was more linear and would require a longer linear number of steps to reach bryostatin 1 than the one that would use a fully functionalized C-ring. However, the full functionalization of the silane **2.144** or similar glycal were not successful and even if it would have worked, that would add too many steps in this piece and would make the synthesis even more linear. Therefore we redesigned our synthesis that would utilize the A-ring as the silane and the C-ring as a fully functionalized aldehyde.

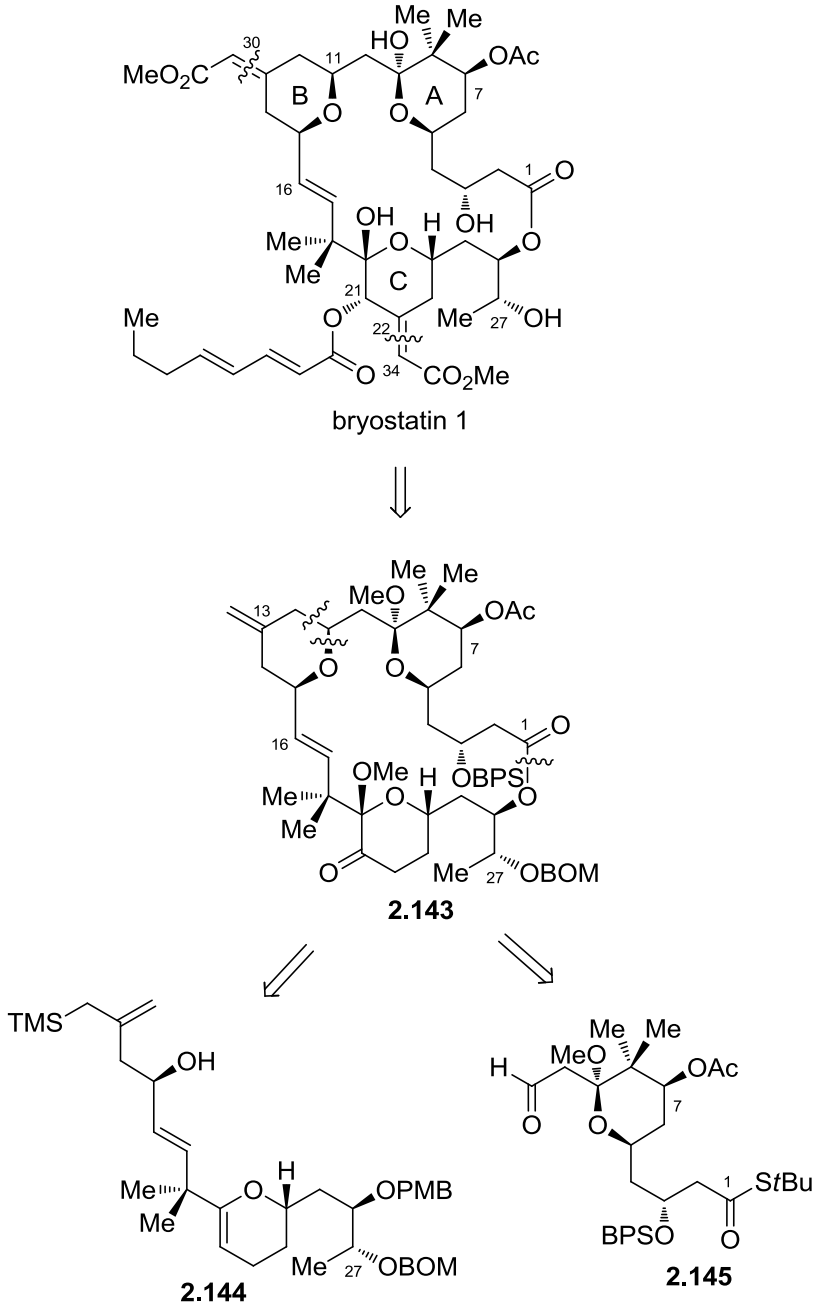


Figure 2.29. First Generation Retrosynthesis of Bryostatin 1

Our new retrosynthetic analysis of bryostatin 1 is shown in Figure **2.30**. This new diagonal disconnection divides the bryostatin into two parts of almost equal complexity, the C-ring aldehyde **2.146** and A-ring β-hydroxy allylsilane **2.147**. The highly convergent assembly of these two fully functionalized fragments would construct the B-ring of the bryostatin with desired stereochemistry in the C_{11} and C_{15} position. In addition, the process would also install an exocyclic olefin handle for the installation of the methyl enoate moiety on C_{13} position. Once the B-ring is formed, the other end of these two fragments would be joined by a macrolactonization reaction.

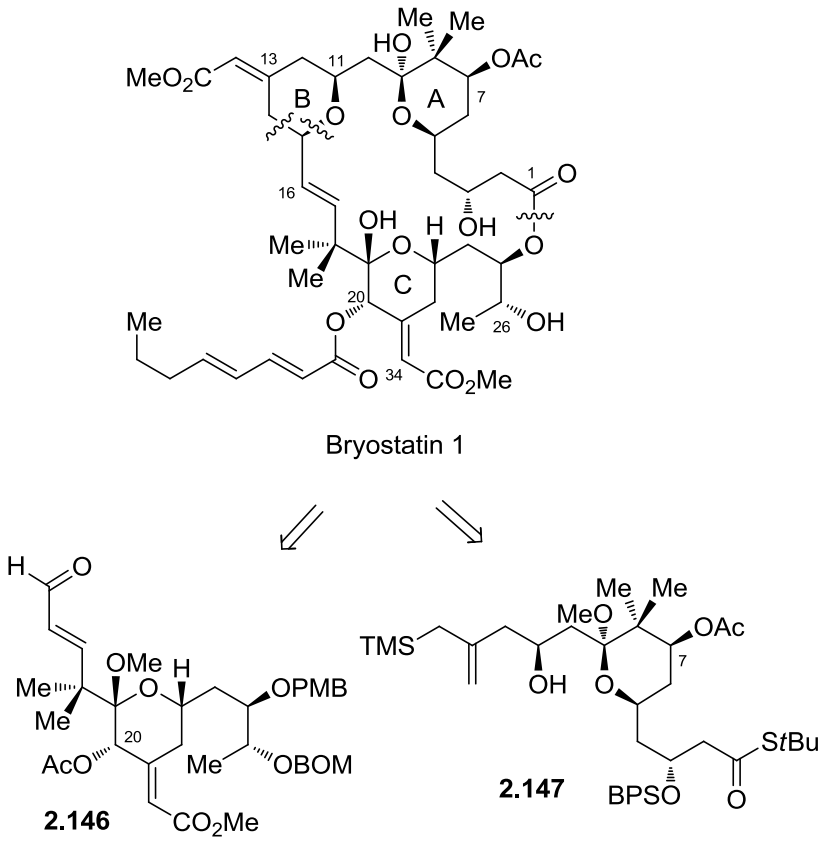


Figure 2.30. Revised Retrosynthetic Analysis of Bryoststin 1

The allylsilane moiety on the A-ring was to be constructed by the addition of trimethylsilyl methylmagnesium bromide to the methylester **2.147** (Figure **2.31**). Formation of the A-ring and installation of the C_9 stereocenter would be achieved from the ketalization/equilibrium of the corresponding keto-alcohol derived from the acyclic precursor **2.148**. The 1,3 antirelationship between the C_5 and C_7 alcohol would allow the use of a chelation controlled addition of stannane **2.149** on the aldehyde **2.150** and set C_7 the stereocenter. This reaction would also install the gem-dimethyl group on the C_8 position. Thus the acyclic precursor **2.148** could be constructed via a highly convergent union of stannane **2.149** and aldehyde **2.150**. The stannane **2.149** would be generated via stannylation of the mesylate of an alcohol ultimately derived from ester **2.151**. The gem-dimethyl group on ester **2.151** was envisioned to be installed by an aldol condensation of the ester **2.153** with acetone. The C_{11} stereocenter was expected to be installed by a catalytic asymmetric allylation (CAA) reaction of corresponding aldehyde. Since C_5 and C_3 stereocenter also have an antirelationship, they could be constructed via another Lewis acid mediated 1,3 chelation controlled addition reaction of thioketene acetal **2.155** on aldehyde **2.154**. The C_5 stereocenter would come from the CAA reaction of the corresponding aldehyde.

In C-ring, the exocyclic methyl enoate would be installed by an aldol condensation of the ketone **2.156** with methyl glyoxylate (Figure **2.32**). The C_{19} stereocenter would be installed by the methanolysis of the glycal followed by equilibration. The C_{20} stereocenter of the fully functionaliozed C-ring aldehyde **2.146** was thought to arise from the reduction of the reduction of the corresponding ketone. The dihydropyran **2.157** was constructed using Rainier modified Takai-Utimoto olefinic ester

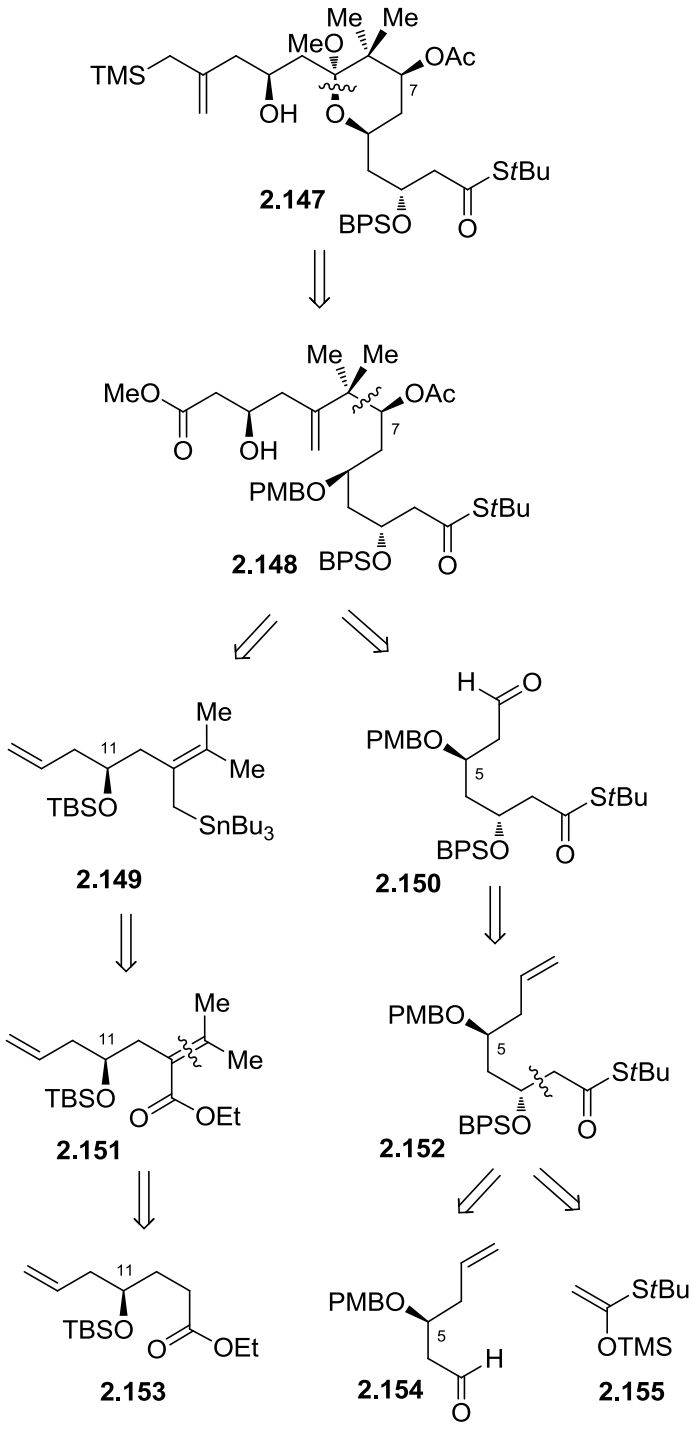


Figure 2.31. Retrosynthesis of A-ring Silane

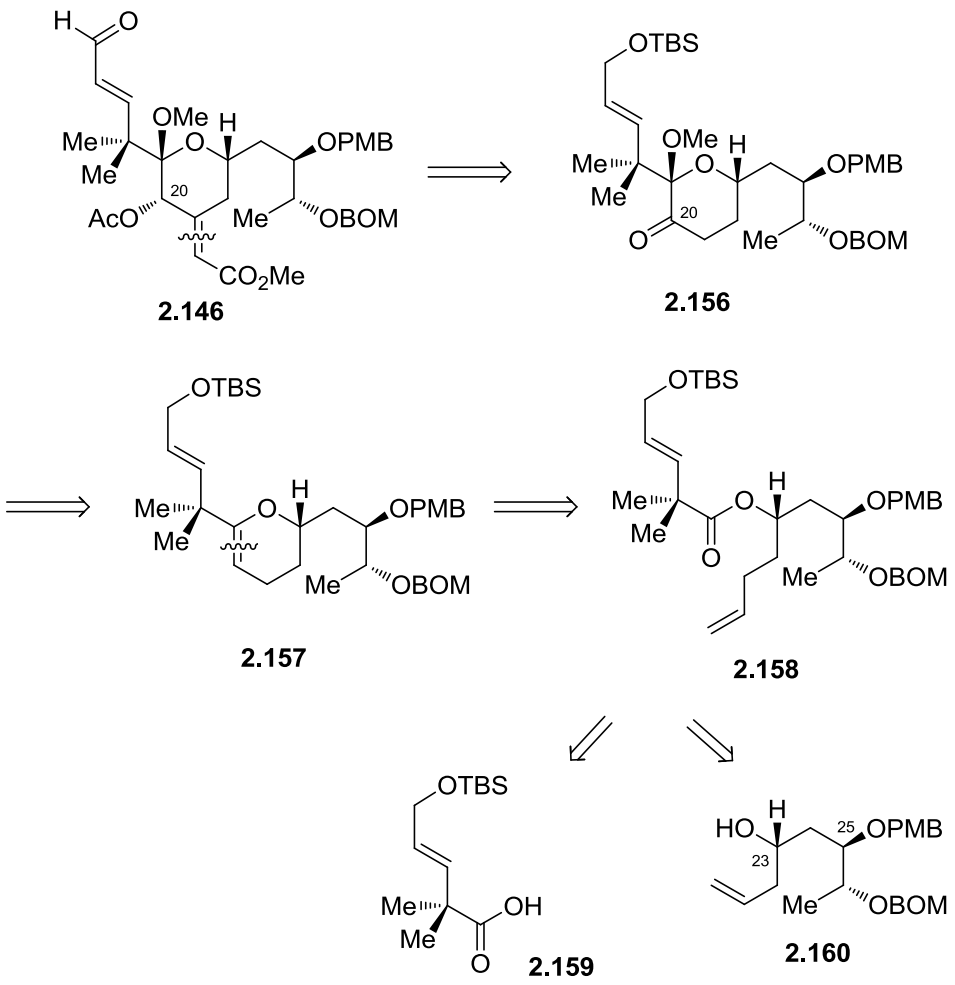


Figure 2.32. Retrosynthesis of the C-ring

cyclization. A highly convergent union of carboxylic acid **2.159** and alcohol **2.160** would be used to synthesize compound **2.158**. The alcohol **2.160** was prepared in six steps from commercially available *R*-isobutyl lactate during which the C_{23} and C_{25} stereocenters were installed through a series of 1,3 and 1,2 chelation controlled allylation reactions. The carboxylic acid piece **2.158** would be formed in four steps from commercially availavle methyl isobutyrate.

First Generation Approach Towards A-ring Silane

Our studies towards the first generation synthesis of the A-ring silane **2.147** was carried out by Dr. Dennie Welch (Figure **2.33)**.[31] The synthesis commenced with the installation of the C_{11} stereocenter using CAA reaction on commercially available aldehyde **2.161**.[32] While the usual CAA reaction takes few days to complete, this particular CAA took only 12 h. This could be rationalized due to the highly electrophilic nature of the aldehyde due to the presence of electron withdrawing group in conjugation with it. The resulting alcohol was protected as a TBS ether and the conjugate reduction of

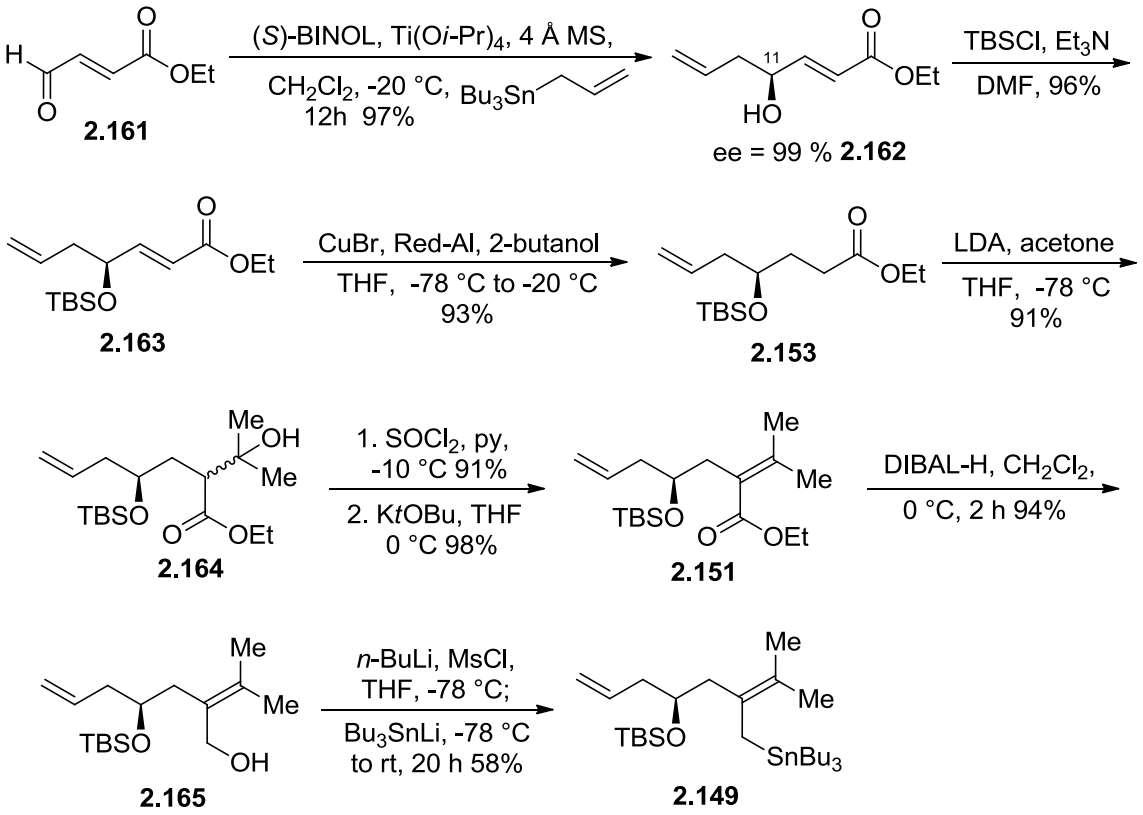


Figure 2.33. Synthesis of Stanne 2.149

the olefin under Semmelhack's condition provided the ester **2.153** in good yield.[33] An aldol reaction of the ester **2.153** with acetone provided the diastereomeric mixture of adduct **2.164** which was eliminated yielding terminal olefins. Migration of the terminal olefin to more substituted position followed by full reduction of the ester provided the alcohol **2.165**. The alcohol was converted into mesylate and in situ displacement with tributyltin lithiate provided the desired stannane **2.149**. The synthesis of the aldehyde **2.150** has been discussed in the synthesis of the bryostatin analogues in Chapter **1**.

With both stannane **2.149** and aldehyde **2.150** in hand, the crucial coupling was attempted (Figure **2.34**). Activation of the aldehyde using five equivalents of dimethyl aluminium chloride followed by addition of the stannane **2.149** provided the alcohol as a single diastereomer. Use of Lewis acids such as $MgBr_2 \bullet OEt_2$ or $TiCl_2(O\textit{i}Pr)_2$ failed to promote the addition whereas use of toluene instead of dichloromethane as a solvent dramatically improved the selectivity. With the successful establishment of the C_7 stereocenter and installation of the gem-dimethyl group, the alcohol was converted into an acetate which is present in the natural product. Attempts to cleave both terminal and internal olefins simultaneously using ozone or $OsO_4/NaIO_4$ were complicated due to various side reactions and failed. Thus a selective osmylation and oxidative cleavage of the terminal olefin followed by Pinnick oxidation provided the carboxylic acid which was converted into its methyl ester using trimethysilyldiazomethane. The PMB group was removed under oxidative condition providing the alcohol **2.169**. Ozonolysis and reductive cleavage of the remaining olefin led to the formation of the keto alcohol which, when treated with acidic methanol, provided the cyclic methyl ketal with concomitant deprotection of the TBS ether.

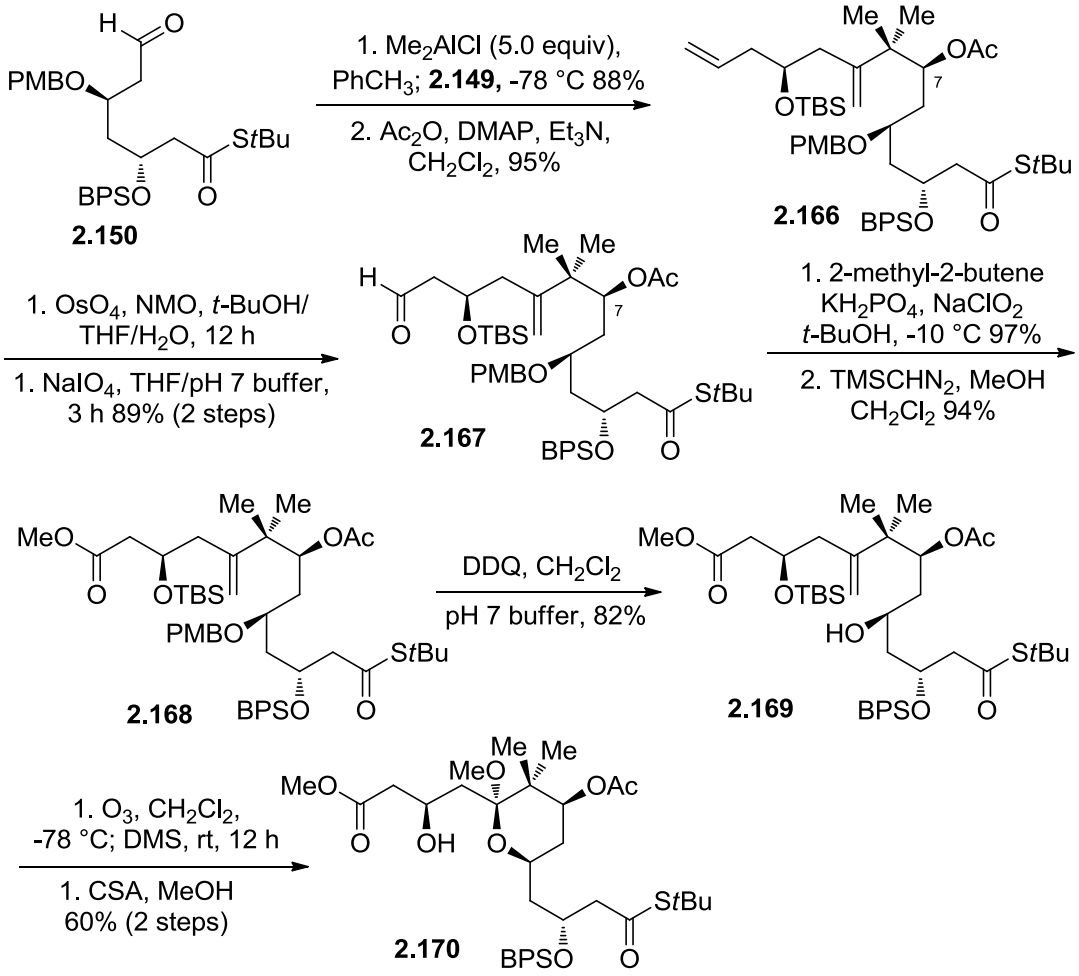
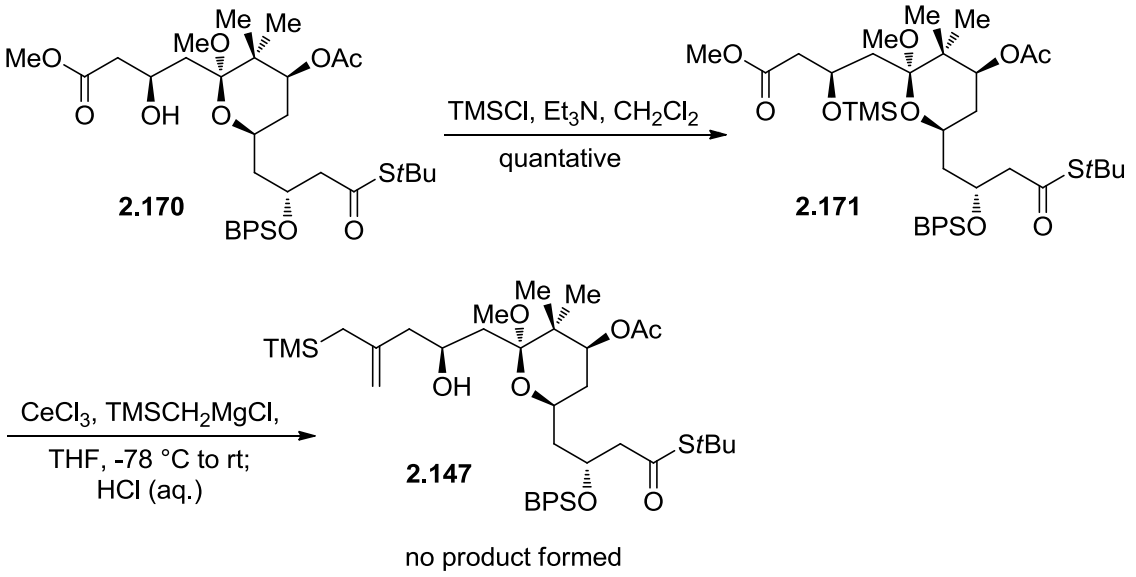


Figure 2.34. Synthesis of A-ring

Temporary protection of the alcohol as a TMS ether prepared the A-ring methyl ester **2.170** for the crucial Bunnelle allylsilane forming reaction (Figure **2.35**).[34] Addition of 15 equivalent of TMS-methylene Grignard reagent to the methyl ester **2.171** in the presence of excess anhydrous Cerium chloride did not provide any desired product. In fact the Grignard reagent did not even react with potentially labile groups such as the methylketal, C_7 acetate ester or C_1 thiolester. Instead, the starting material in which TMS



Scheme 2.35. Attempted Synthesis of A-ring Silane

group was deprotected was fully recovered without any by products. The reaction was also attempted under reflux condition with or without cerium chloride and still the starting material was left intact. The possibility of the fact that Grignard reagent might be bad was ruled out by preparing fresh batches of the reagent every time and testing it with simple methyl esters such as methyl benzoate. While the model substrate provided the desired allylsilane in high yield, the ester **2.171** gave no desired product under the identical conditions.

Failure to form the desired allylsilane from the ester **2.171** led us to assume that steric factors could be keeping the ester from reacting with the Grignard reagent. One such sterically hindering group could be the TMS ether. With this in mind, the reaction was attempted with a methylester **2.170** with free alcohol in the beta position. In fact one

mole of the Grignard reagent reacted with the methyl ester and the resulting TMS methylene ketone was isolated. Subjection of the ketone to excess Grignard reagent did not yield any desired product. It was hypothesized that the cyclized A-ring might be imposing the steric hindrance for the addition of the Grignard reagent. To test this hypothesis, the open chain methylester **2.172** in which the TBS group was switched for a TMS group was subjected to Bunnelle reaction (Figure **2.36**). Once again, the reaction did not provide any desired product but the starting material. However, the methylester with free alcohol gave the TMS-methylene ketone which failed to react further. At this point, it is not known which factor is responsible for the inherent inertness of the methyl ester but the steric hindrance caused by the gem-dimethyl group coupled with various other functional groups seems to be a probable reason.

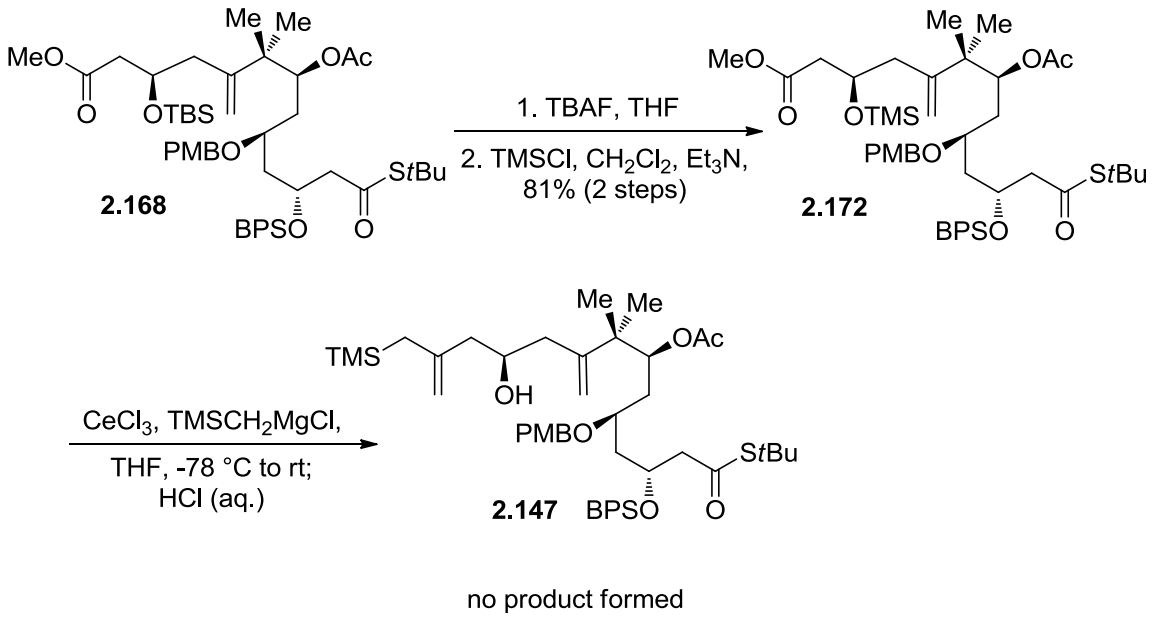


Figure 2.36. Attempted Synthesis of A-ring Silane

An Alternative Synthesis of A-ring Silane

Since the formation of the desired A-ring hydroxyallylsilane from the methyl ester **2.171** was found impossible to work, alternative routes to such silane were sought. One such route involved a CAA reaction of the trimethylsilyl methylallylstannane to an aldehyde to construct the *β*-hydroxyallylsilane. The A-ring aldehyde **2.173** was prepared in multigram quantities and was successfully used in the synthesis of various bryostatin analogues. A catalytic asymmetric allylation (CAA) reaction on such aldehyde would set the C_{11} stereocetner as well as install the allylsilane moiety (Figure **2.37**). Unfortunately, when the reaction was attempted using various CAA conditions such as high catalyst loading, warming to room temperature etc. and no desired product but starting material was isolated. As the substrate is highly oxygenated, it was suspected that the catalyst might undergo competitive binding with these functionalities and thus not activate the aldehyde enough for nucleophilic addition. Addition of super stoichiometric amount of BITIP catalyst did not have any effect and only starting material was recovered. Use of Lewis acids such as $MgBr_2 \bullet OEt_2$ was found to be ineffective whereas stronger Lewis acids such as $BF_3 \bullet OEt_2$ resulted in the decomposition of the starting material.

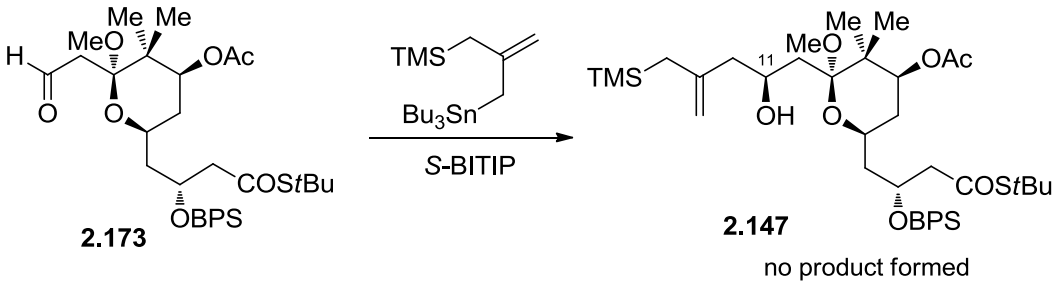
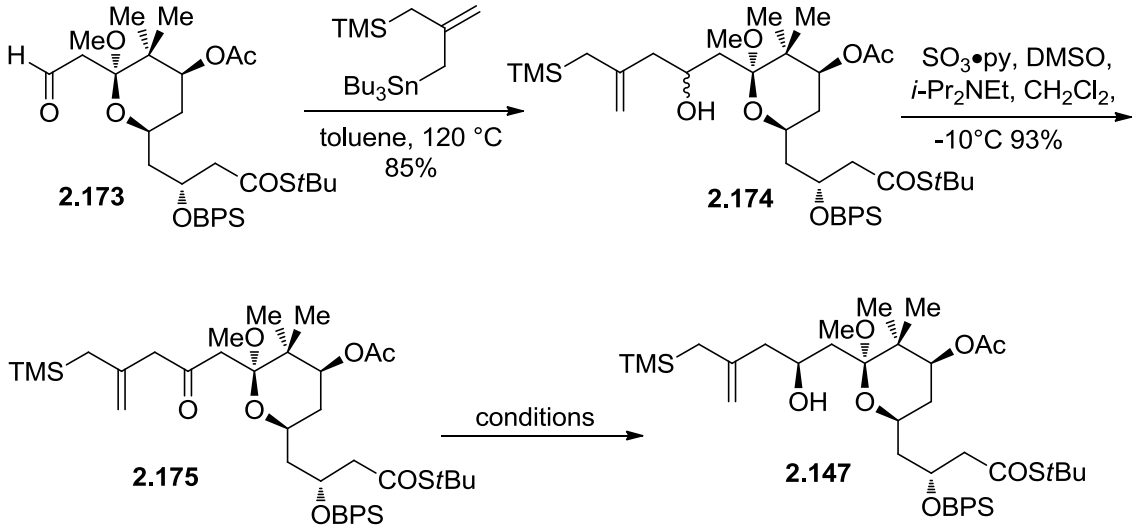


Figure 2.37. Attempted Alternate Synthesis of Silane 2.147

As the asymmetric synthesis of the hydroxy allylsilane **2.147** directly from aldehyde **2.173** failed, an alternative approach was undertaken (Figure **2.38**). Thus a thermal addition of the trimethylsilyl methallyl tributylstannane to the aldehyde **2.173** produced the 1:1 mixture of hydroxyallylsilane **2.174**. Initial attempts to separate the mixture of diastereomers using silica gel column chromatography under numerous solvent systems failed. Although it was found that the mixture could be separated using preparative thin layer chromatography eluting with with 1% EtOAc in benzene, the separation proved to be impractical on large scale. Alternatively, the mixture of alcohols was oxidized to ketone **2.175** and was envisioned to asymmetrically reduce to an alcohol either using a chiral reagent or using substrate directed reduction. Use of a external chiral reagent such as the CBS catalyst was ineffective.[35] Despite the absence of any literature precedence, it was anticipated that the pyran oxygen could direct a 1,3 chelation assisted reduction of the ketone. Samarium iodide is known for 1,3 *anti* or sometimes *syn* reduction to beta keto alcohols and has been successfully used in the total synthesis of Epothilone.[36] Unfortuantely, the ketone **2.175** was completely inert to samarium iodide reduction. Application of Evans-Saksena reaction did not provide any product and the starting material was recovered.[37] A metal hydride with stronger chelating ability such as $ZnBH_4$ was also ineffective. Other hydrides such as $LiBH_4$, DIBAL-H and $LiAlH_4$ resulted in the formation of 1:1 diastereometic mixture of alcohols along with reduction of the thioester and acetates.

The metal hydrides used thus far were either not able to reduce the ketone or also reduced the esters with no selectivity in either case. However, when the reduction was attempted with $NaBH_4$, only the reduction of the ketone was observed with no selectivity.



Reduction Conditions	Results
SmI_2/MeOH, -78°C to rt	no reaction
$Me_4N^+(AcO)_3BH^-$, -78°C to rt	no reaction
$ZnBH_4$, THF, -78°C to rt	no reaction
$LiBH_4$, THF, -78°C to 0 °C	dr = 1:1 thioester reduction
CBS, CH_2Cl_2, -78°C to rt	no reaction
$LiAlH_4$, THF, -78°C	dr = 1:1 both ester reduction
DIBAL-H, THF, -78°C	dr = 1:1 both ester reduction
$NaBH_4$, MeOH -78°C to 0 °C	63 %, dr = 1:1
$CeCl_3$/$NaBH_4$, MeOH -78°C to 0 °C	82%, dr = 4:1

Figure 2.38. Alternative Synthesis of A-ring Silane 2.147

Cerium chloride is an additive known to chelate the ketone in the Luche reduction of α,β-unsaturated ketones and it was thought that it could probably chelate with both the pyran and ketone oxygens.[38] In the event, we were astonished to find that reduction of the ketone **2.175** under Luche condition in fact afforded the alcohol in good yield with moderate selectivity. Attempts to improve the selectivity of the reduction by maintaining the reaction temperature at -78°C or -42°C did not have any effect and the reaction did not go to completion. At the present moment, the origin of the selectivity of the ketone reduction under Luche condition is not fully understood, but the chelation of both pyran and ketone oxygen by cerium and an intermolecular hydride delivery from the *beta* face seems to be the most logical explanation.

With the successful reduction of the ketone, verification of the stereochemistry of the alcohol **2.147** using a chemical method was addressed next (Figure **2.39**). The A-B-ring compound **2.177** had been prepared and characterized previously (Figure **2.39-a**).[39] A pyran annulation of the hydroxy allylsilane **2.147** with the aldehyde **2.178** would give the same compound **2.178** and their NMR could be compared. Unfortunately, the reaction with aldehyde **2.178** failed and no desired product was isolated. Therefore, a similar approach was adjusted whereby the diastereomerically pure bispyran compound **2.180** was prepared using a pyran annulation reaction between the aldehyde and a known silane **2.179**. Another pyran annulation using the 4:1 mixture of hydroxyallylsilane **2.147** and hydrocinnamaldehyde **2.181** provided the compound **2.182**. It should be noted that although the compound **2.182** has two unknown stereocenters with a possibility of 4 diastereomers, only two isomers can result in which both substituents at 2 and 4 positions are equatorial. This is due to the fact that the pyran annulation proceeds in such a way

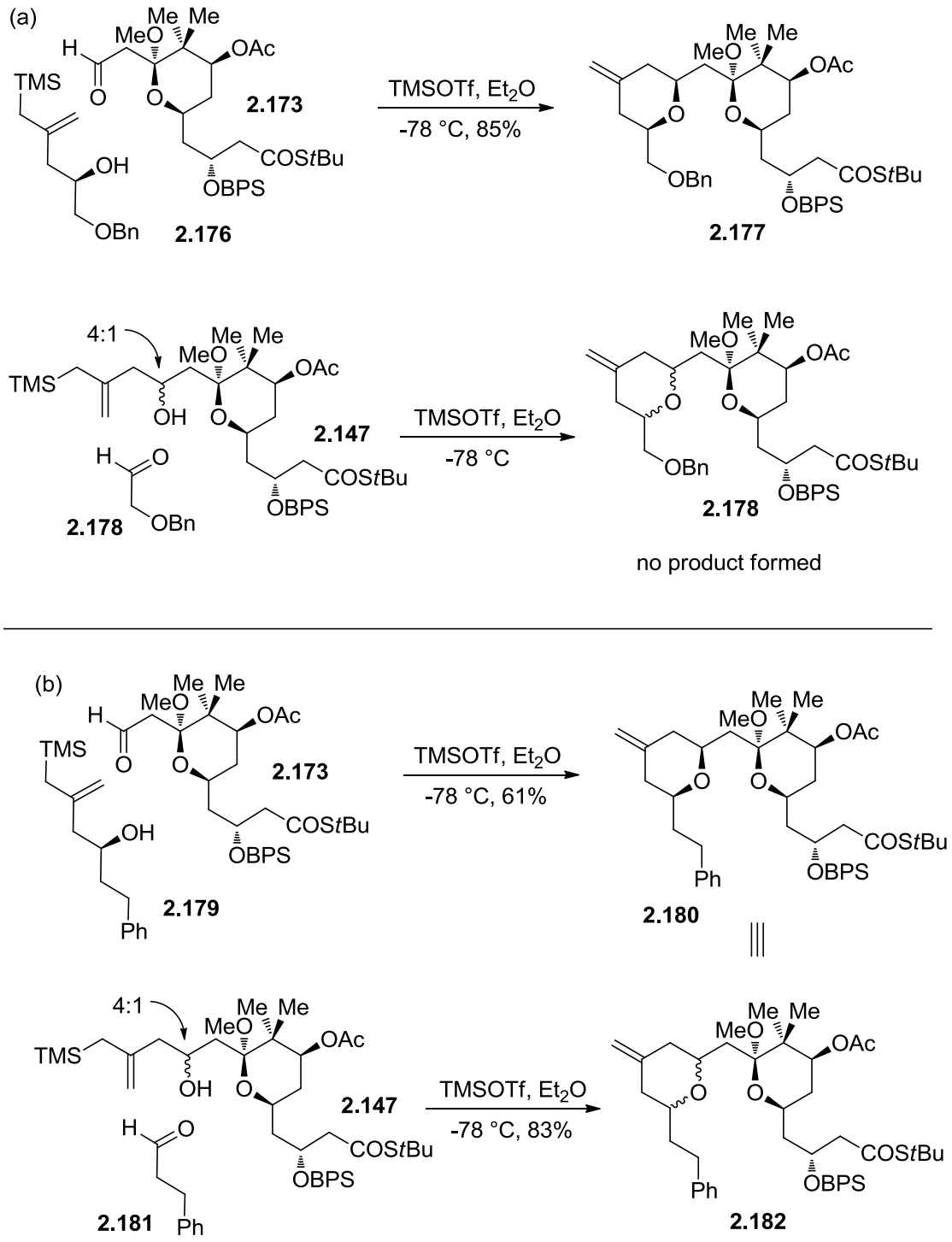


Figure 2.39 Verification of the C_{11} Stereochemistry

that the substituents at the 2 and 4 positions always occupy the equatorial position. The ^{1}H and ^{13}C NMR of the major isomer of bispyran **2.182** matched with that of authentic compound **2.180** thus confirming that the major isomer of the alcohol **2.147** had the desired stereochemistry. The synthesis of A-ring hydroxy allylsilane **2.147** was thus completed in 17 steps (longest linear sequence) from aldehyde **2.173**. The synthesis is based on the substrate controlled generation of stereocenters and required only one chiral material to install the C_5 stereocenter. All remaining four stereocenters were installed with the help of the C_5 stereocenter in a highly substrate controlled manner.

A Convergent Synthesis of C-ring Aldehyde

With the preparation of the A-ring hydroxyallylsilane **2.147** accomplished, we focused our attention toward the synthesis of the C-ring aldehyde **2.146** (Figure **2.40**). This required the preparation of glycal **2.157** which could be derived from the thioester **2.184** used during the synthesis of bryostatin analogues. Although the glycal **2.157** could be prepared in gram scale, frequent requirement of this material in multigram quantities for all bryostatin projects demanded a more concise synthesis. The only real drawback in the route to the dihydropyran **2.157** was the linear nature of its synthesis. Thus any improvement in the existing route would require a convergent approach for the synthesis of this material.

The presence of the glycal moiety in the intermediate **2.157** led us to consider its formation by a more direct method instead of the existing route that involves the dehydration of the hemiketal derived from a keto alcohol. One of the most versatile

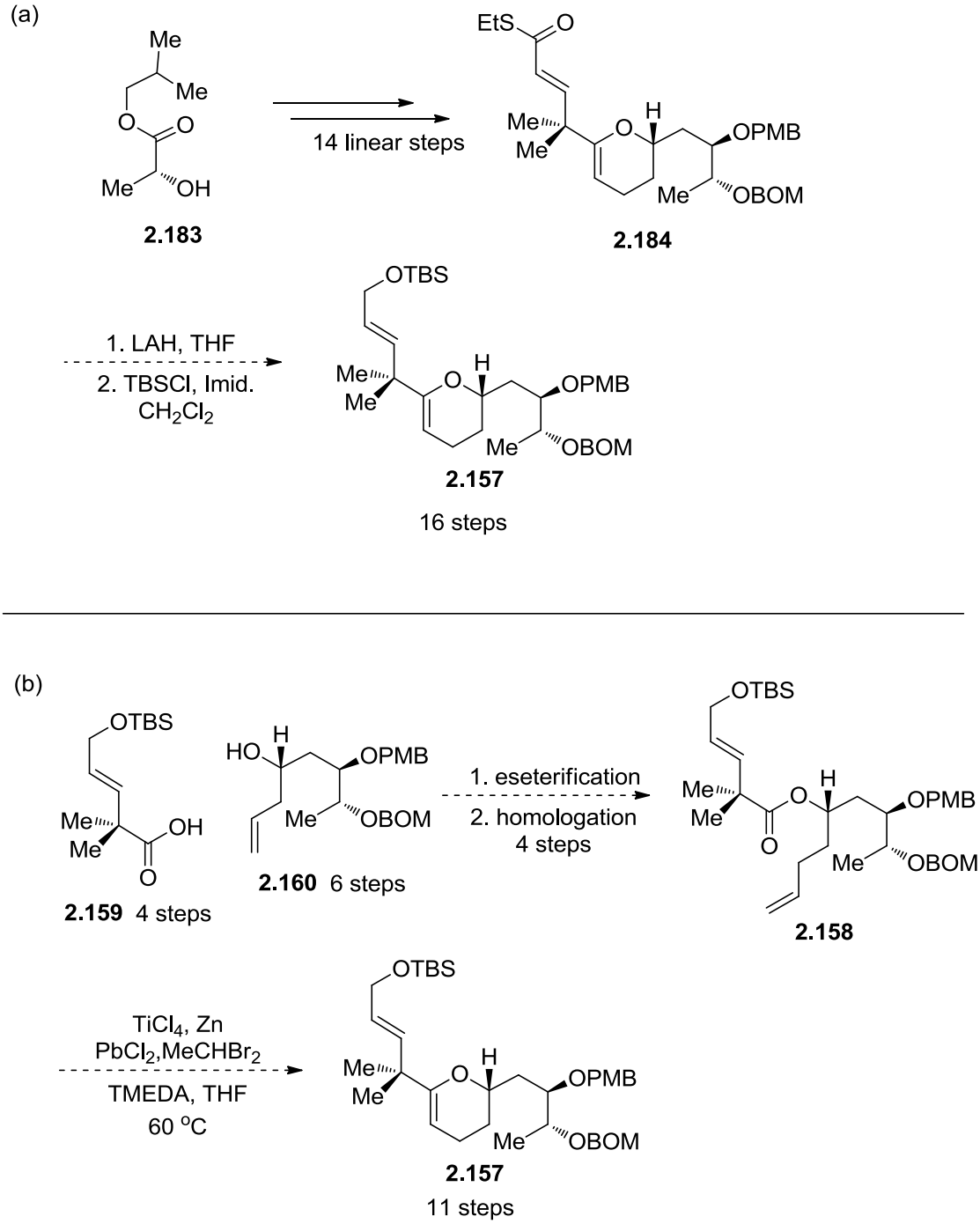
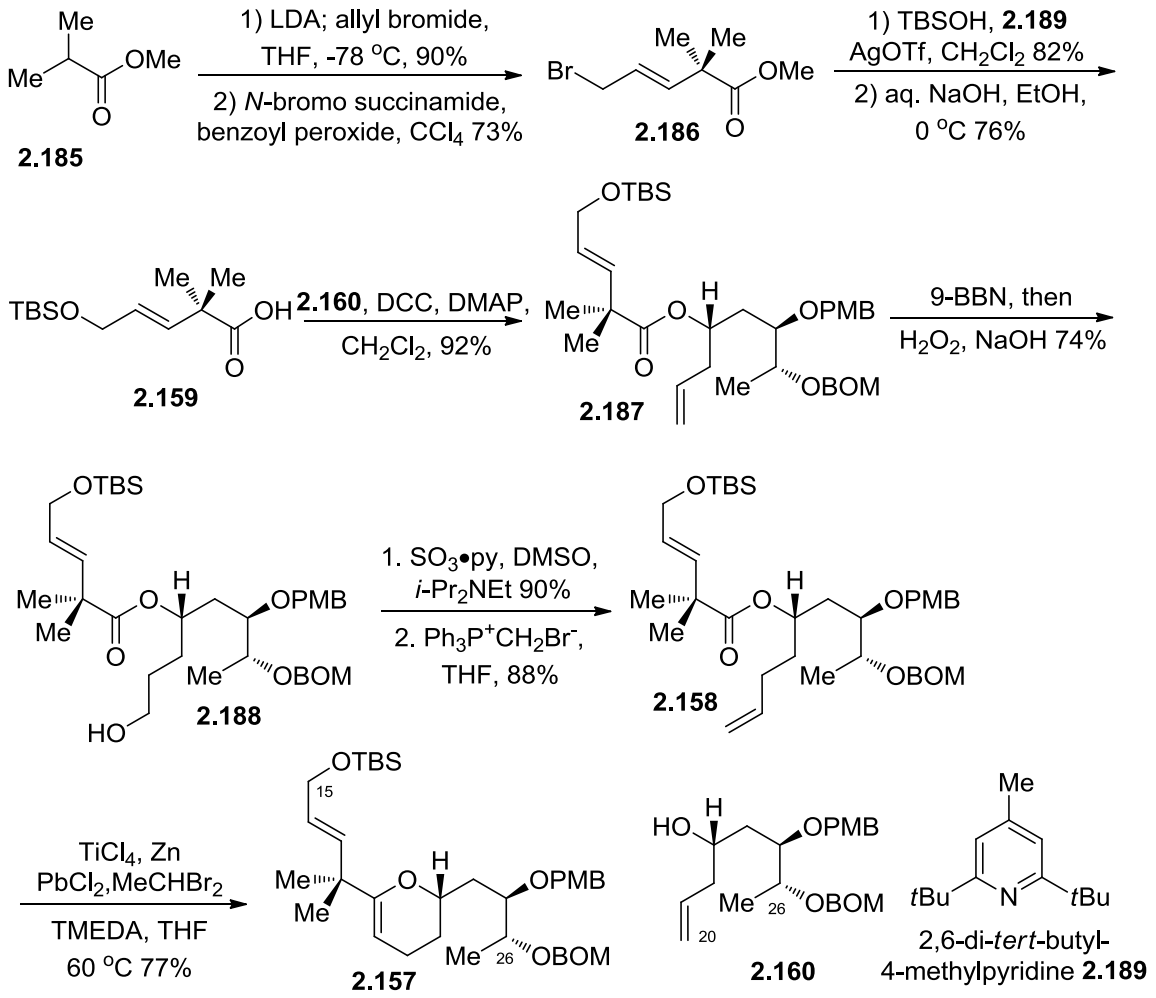


Figure 2.40. Comparison of Two Routes Towards Glycal 2.157 (a) old, (b) new

methods used to generate the cyclic enol ether is the Takai-Utimoto ester olefin cyclization and has been successfully used during the total synthesis of various natural products.[40] An important modification to the original Takai-Utimoto reaction was developed by Professor Jon D. Rainier at the University of Utah in which they use dibromoethane instead of dibromomethane.[41] Use of this alternative halide dramatically increased the ratio of cyclic versus acyclic enol ether products. If we could apply this Rainier metathesis reaction in the synthesis of the dihydropyran **2.157**, this would make use of ester **2.158** as a precursor. Such an ester could be easily generated by a convergent esterification using a relatively simple alcohol and acid. Moreover, the alcohol fragment **2.160** was prepared in just six steps during the synthesis of bryostatin analogues. This new approach would not only signigicantly reduce the number of steps to achieve glycal **2.157**, but also make the synthesis highly convergent.

Approach towards the synthesis of glycal **2.157** was developed by another graduate student Thomas J. Cummins and by the author.[42] Briefly, the acid fragment **2.159** was synthesized in four steps from commercially available methyl isobutyrate **2.185** (Figure **2.41**). Alkylation of the ester **2.185** followed by a free radical bromination provided the allyl bromide **2.186** . The bromine was displaced with *t*-butyldimethylsilanol in the presence of silver triflate. Basic hydrolysis of the methyl ester furnished the acid fragment **2.159** which was esterified with the previously known alcohol **2.160**. The olefin was subjected to hydroboration/oxidation reaction and the resulting alcohol was oxidized to an aldehyde. A one carbon homologation of the aldehyde using Wittig reaction prepared the olefin. Subjection of the ester olefin **2.158** to Rainier metathesis reaction provided the desired glycal in 77% yield.



Scheme 2.41. Synthesis of C_{15}-C_{27} Subunit 2.157

Difficulties associated with the late stage functionalization of the C-ring in tricyclic compound during the synthesis of bryostatin analogous led us to consider functionalization of it before pyran annulation. Of the most difficult reactions during analogue synthesis was the aldol reaction between the C-ring ketone and methyl glyoxylate (Figure **2.42**). This was due to a competitive aldol reaction taking place on the C_7 acetate of the A-ring and could be eliminated if the aldol reaction could be carried out

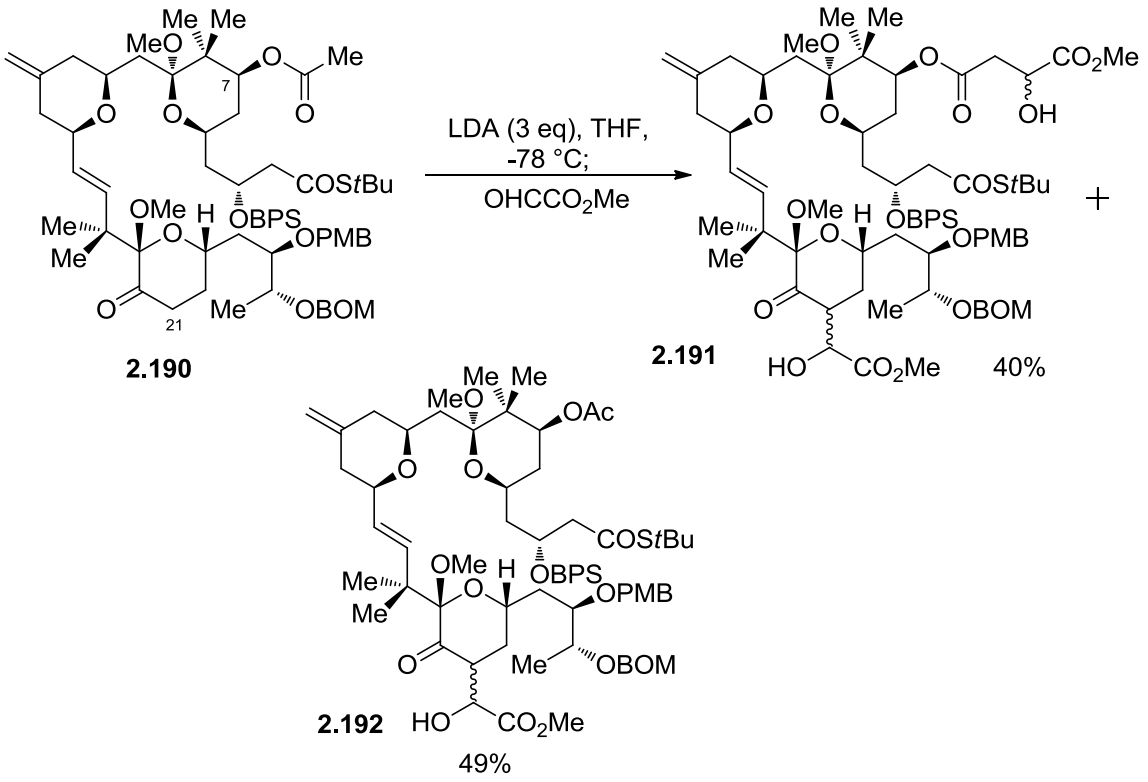


Figure 2.42. An Unselective Aldol Reaction

on a C-ring compound prior to coupling with A-ring silanes. Such an early functionalization would also make the synthesis highly convergent. Thus the epoxidation of the glycal **2.157** using MMPP followed by *in situ* opening with methanol furnished a methoxy alcohol compound which was immediately oxidized to a ketone using Ley oxidation (Figure **2.43**).[43] An aldol condensation between the ketone **2.156** and freshly distilled methylglyoxyolate provided the α,β-unsaturated ester **2.193** as single geometrical isomer in excellent yield. The ketoester **2.193** possessed a characteristic bright yellow color and was found to be unstable for long term storage or for carrying this functional group along during the synthesis. Therefore, the ketone was reduced using

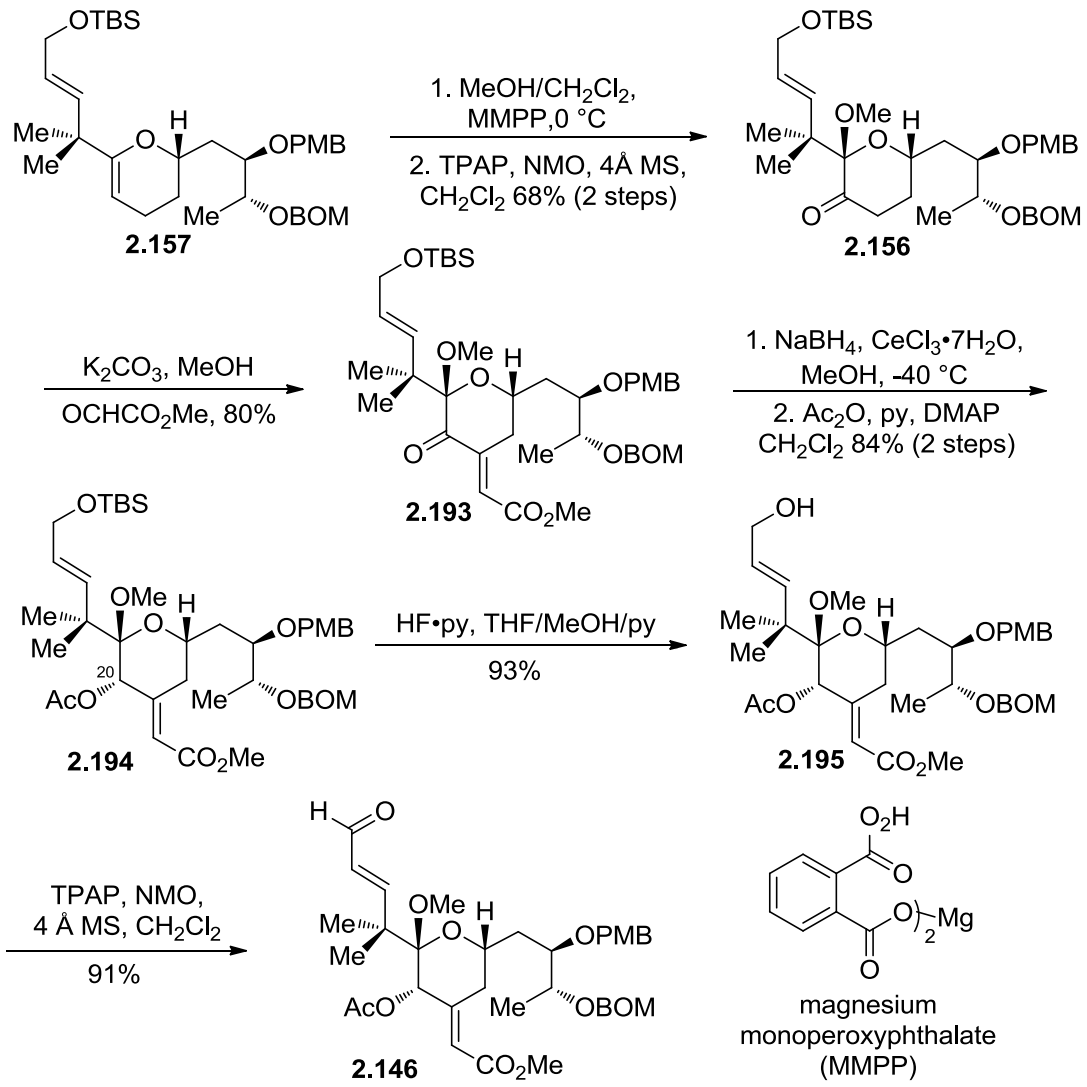


Figure 2.43. Synthesis of Fully Functionalized C-ring Aldehyde 2.146

Luche condition providing the alcohol as single diastereomer which was also found unstable for purification using silica gel chromatography. This required the protection of the alcohol with some protecting group. Conversion of the alcohol into an ester having the α,β-unsaturated bryostatin side chain was not applicable because it could not be differentiated from the B-ring olefin for the oxidative cleavage step that would be required. Attempted conversion of the alcohol into a silyl ether using TBSCl was

unsuccessful whereas use of TESOTf provided a low yield of the desired product due to the sensitive nature of the alcohol starting material. Use of protecting groups such as trifluoroacetate or chloroacetate provided the desired esters but the esters turned out to be unstable for subsequent transformations. Hoping that the C_{20} and C_7 acetate could be differentiated later in the synthesis, the C_{20} alcohol was converted into its acetate. Removel of the TBS group under standard condition followed by oxidation of the resulting alcohol completed the synthesis of fully functionalized aldehyde in 18 steps (longest linear sequence).

During the synthesis of Merle 32 it was observed that the basic methanolysis of the C_7 acetate was slow and took 6 h to complete, but occured without any side reactions such as transesterification of the macrolactone ester (Figure **2.44**). When the acetate **2.194** was subjected to the same reaction condition, interestingly, the acetate group was removed in just 1 h indicating that the C_{20} acetate was at least five times more reactive than the C_7 acetate. The difference in the reactivity of these two acetates is presumably due to the activation of the C_{20} acetate by an inductive effect of the surrounding groups. Thus the conversion of C_{20} alcohol into its acetate might permit its selective removal in the presence of the C_7 acetate for the synthesis of bryostatin 1 (*vide infra*). Moreover, both the acetates could be kept for the synthesis of bryostatin 7. If this selective removal of C_{20} acetate is successful, this would allow the synthesis of other members of the bryostatin family that have acetate at C_7 position and different esters at the C_{20} position. With this idea in mind, it was decided to use acetate as protecting group for the C_{20} acohol.

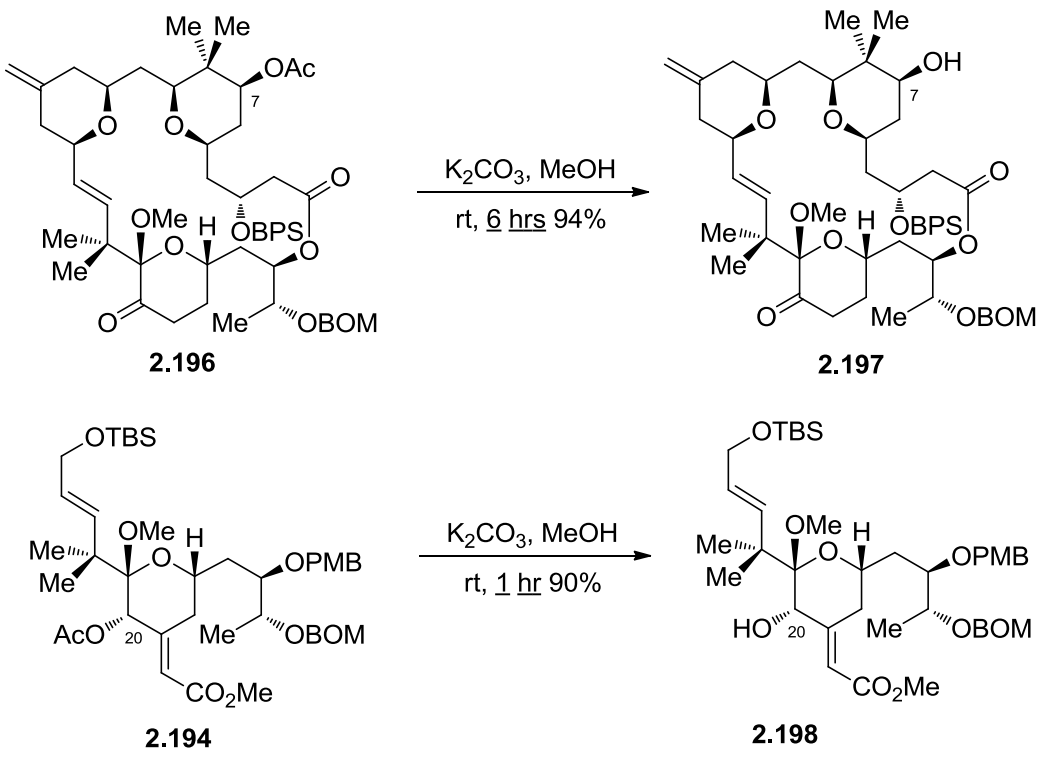


Figure 2.44. Model Methanolysis of the Acetates

Coupling of the A and the C-ring and the Completion of Bryostatin 1

With both fragments in hand, A-ring hydroxyallylsilane **2.147** and and C-ring aldehyde **2.146** were subjected for the crucial pyran annulations (Figure **2.45**). The reaction provided the tricyclic compound **2.199** in good yield. Along with the usual side reactions such as TMS protection of the hydroxyl group and protodesilation of the allylsilane, few other side reactions were noticed. The major by product was found to result from an inramolecular cyclization of the hydroxy allylsilane to the C_9 position leading to the formation of a spirocyclic compound. Attempts to isolate this side product were not possible due to overlap with other byproducts but it could be detected in mass

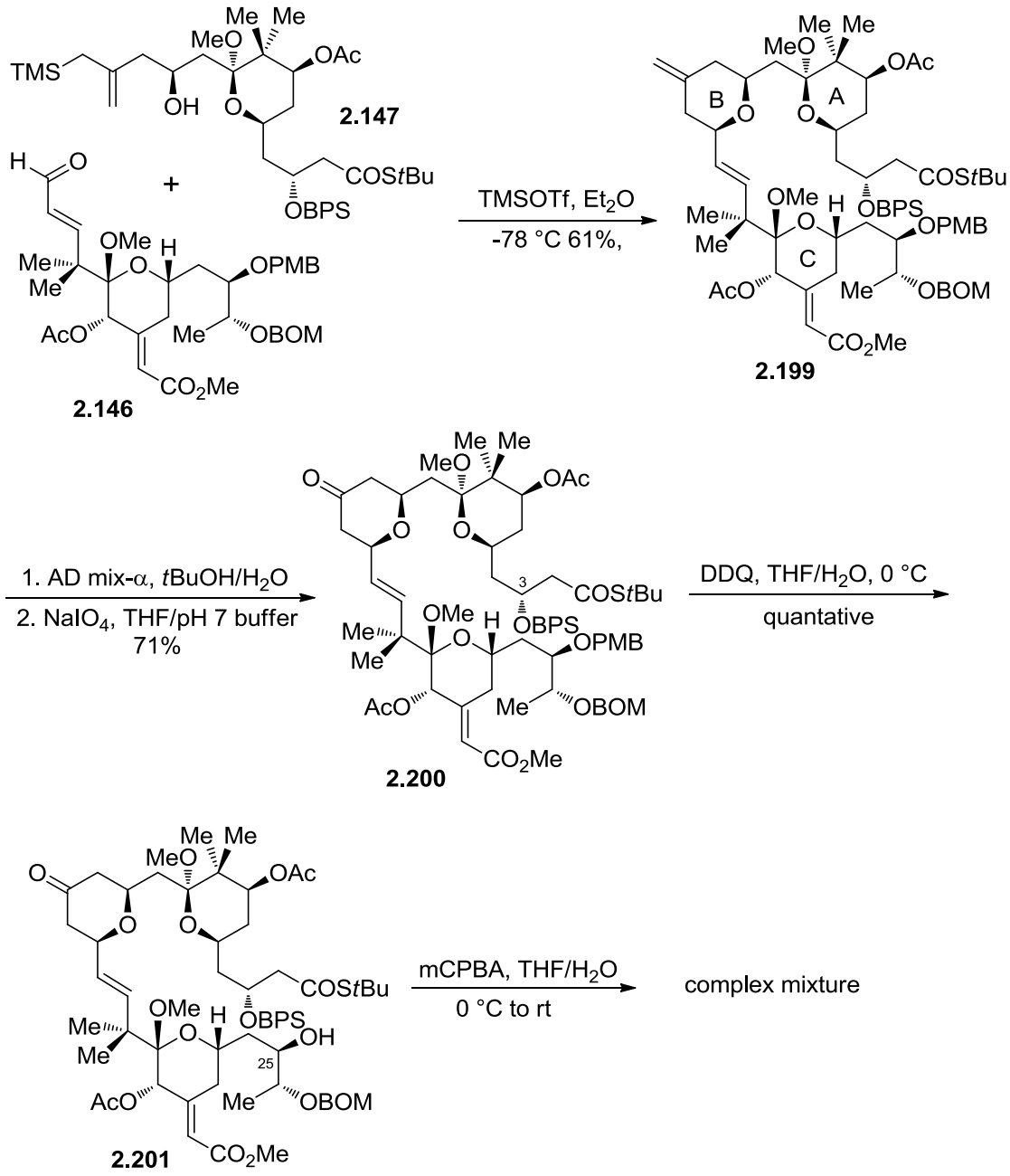


Figure 2.45. Attempted Preparation of the Seco Acid

spectrum. Efforts to eliminate the intramolecular cyclization of the allylsilane by concentrating the reaction mixture or increasing the number of equivalents of the aldehyde partner were fruitless. Partial decomposition of the aldehyde **2.146** and silane **2.147** was also noticed if the temperature of the reaction was increased to 40 °C or above.

Advancement of the tricycle **2.199** required the regioselective cleavage of the exocyclic B-ring olefin in the presence of an internal olefin and the exocyclic olefin on the C-ring. Studies during the synthesis of bryostatin analogue Merle 30 showed that ozonolysis or osmylation were unselective for these two olefins. However, the exocyclic olefin could be cleaved selectively by carefully treating the bisolefin with a solution of ozone followed by dimethyl sulfide.[29] When these conditions were applied to bisolefin **2.199**, the reaction was completely unselective. It should be noted that the major difference between those two substrates is the presence of an extra methoxy group at the C_9 position and a full functionalized C-ring in **2.199**. The reason for the failure to selectively ozonize the exocyclic olefin in the compound **2.199** is not fully understood but it is most likely due to the blockage of the exocyclic olefin by the C_9 methoxy group. However, it was later found that the C_{13}-C_{30} olefin could be selectively oxidized to a diol using Sharpless reagent.[44] Thus, the regioselective dihydroxylation of the B-ring under Sharpless condition followed by oxidative cleavage provided the ketone **2.200** in good yield. Either AD mix-α or AD mix-β were equally effective for the dihydroxylation reaction. Removal of the PMB group under oxidative conditions provided the alcohol **2.201**. Subjection of the thioester **2.201** to hydrolysis conditions using *m*CPBA in THF/water gave a complex mixture of products which lacked the S-*t*Bu signal in the ^{1}H-NMR. The proton NMR also showed the absence of the C_9 methoxy group indicating that

the hydrolysis of the methylketal had also occurred. Attempts to convert the mixture of products into a single compound and to restore the C_9 methylketal using PPTS/MeOH or CSA/MeOH led to complete decomposition.

Since the hydrolysis of the thioester under slightly acidic conditions using *m*CPBA in THF/H_2O was unsuccessful, an alternative route towards the seco acid was sought (Figure **2.46**). During the synthesis of bryostatin analogue Merle 28, it was

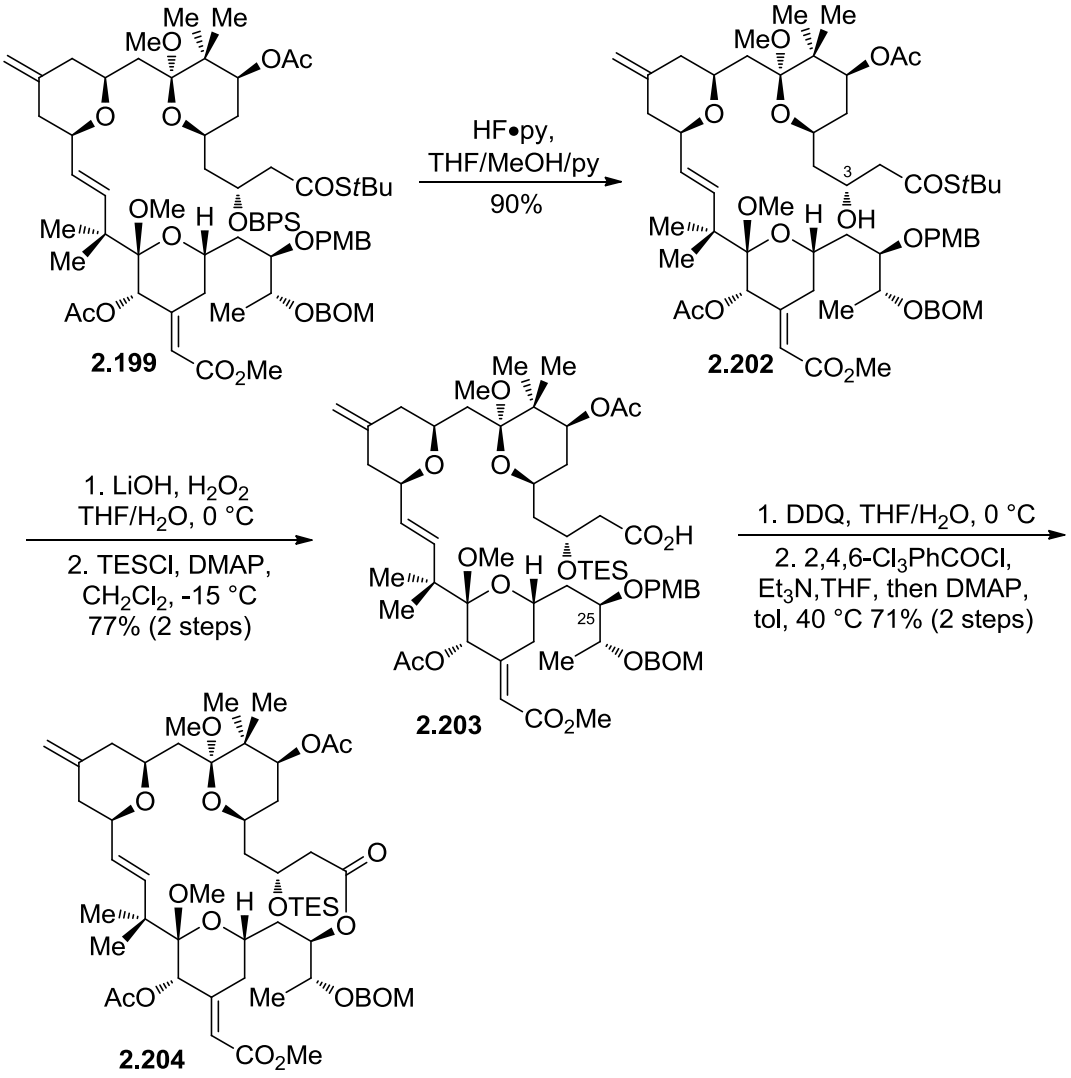


Figure 2.46. Synthesis of Macrolactone 2.204

discovered that $LiOH/H_2O_2$ mediated thioester hydrolysis could be carried out in the presence of other esters and the C_9 methylketal in the presence of free C_3 alcohol.[28] Thus, the BPS group at C_3 position as removed using buffered HF•py in THF/methanol. Use of methanol during the BPS deprotection was necessary to avoid the hydrolysis of C_9 and C_{19} methylketals. Hydrolysis of the thioester **2.202** took place smoothly using $LiOH/H_2O_2$ providing the hydroxy acid intermediate. Treatment of such hydroxy acid with TESCl protected both the alcohol and the carboxylic acid and selective removal of the TES ester occurred during workup and silica gel column chromatography providing the carboxylic acid **2.203**. The PMB group was removed using DDQ providing the seco acid which was subjected to Yamaguchi esterification furnishing the macrolactone **2.204**.

With the macrolactone in hand, Sharpless asymmetric dihydroxylation of the bisolefin regioselectively provided the exocyclic diol which was oxidatively cleaved to ketone **2.205** (Figure **2.47**). An asymmetric Horner-Wadsworth-Emmons reaction on the ketone using Fuji's chiral BINOL phosponate **2.206** provided a 4:1 mixture of *Z*:*E* α,β-unsaturated methyl esters in favor of the desired isomer.[45] The geometric isomers were easily separated using preparative thin layer silica gel chromatography using 10% EtOAc in benzene and eluting twice. When the bisacetate **2.207** was subjected to K_2CO_3/MeOH, we were pleased to find that the selective methanolysis of the C_{20} acetate occurred in just 45 min providing the desired alcohol, which proved to be unstable for purification and characterization. Threfore, it was immediately esterified with (2*E*,4*E*)-octa-2,4-dienoic anhydride providing protected bryostatin 1. When the protected bryostatin 1 **2.208** was subjected to global deprotection using $LiBF_4$ in acetonitrile/water at 80 °C, two

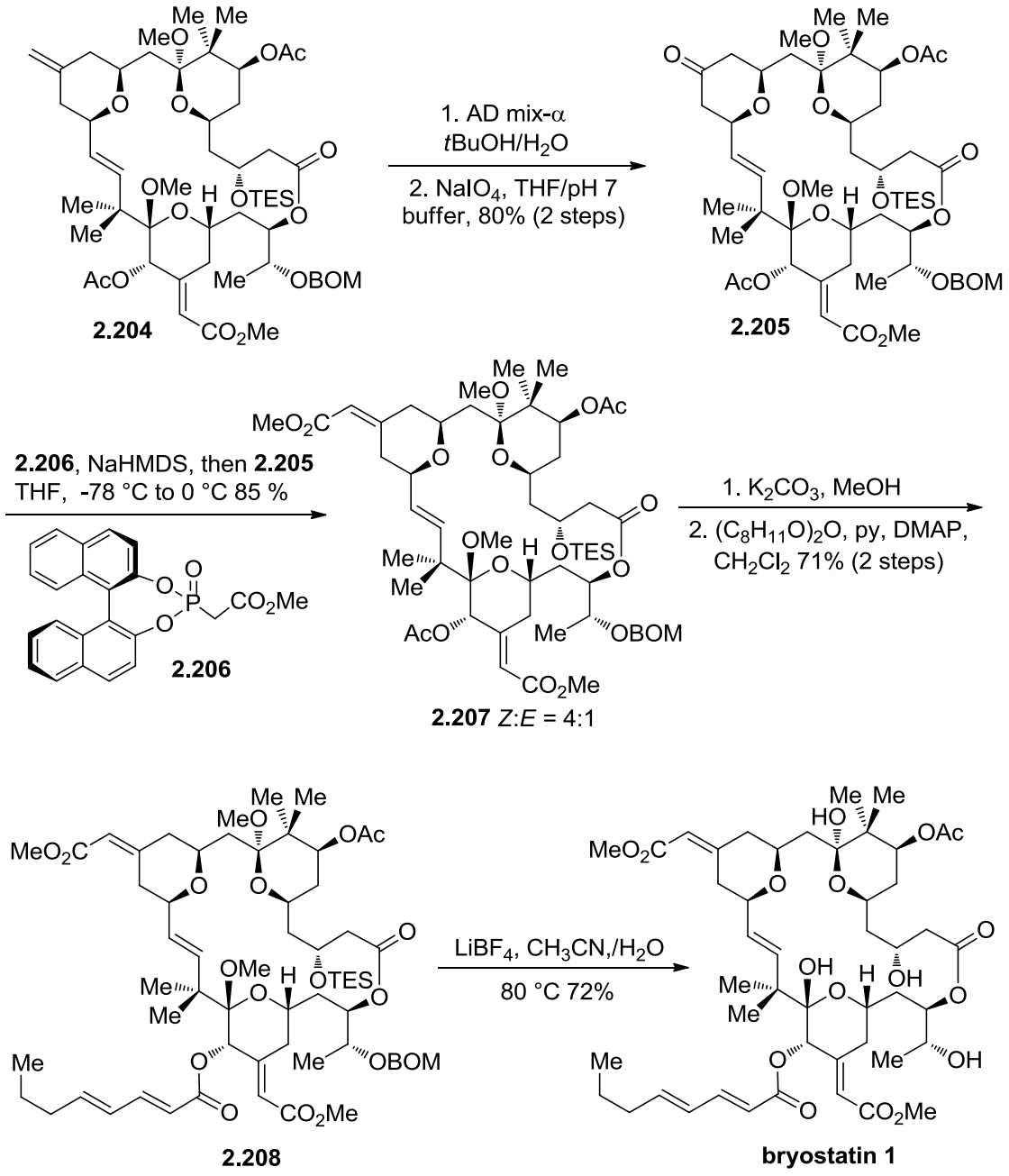


Figure 2.47. Completion of the Total Synthesis of Bryostatin 1

methyketals and a TES group were removed in just 1 h.[46] Further heating the mixture for 12 h removed the BOM group leaving the other five esters untouched and provided bryostatin 1 in 72% yield.

The synthetic bryostatin 1 was found identical to the natural bryostatin 1 in several criteria such as TLC, ^{1}NMR, ^{13}CNMR, ^{13}C DEPT, high resolution mass and the optical rotation. As seen in the literature[47] and by us, the NMR of the bryostatins is highly concentration dependent, presumably due to hydrogen bonding with the solvent. The concentration effect is more prominent especially when any amount of H_2O or D_2O is present in the NMR solvents. Thus the comparison of the NMR of synthetic and natural bryostatins was carried out under similar concentrations (Figure **2.48** and **2.49**).

In addition to bryostatin 1, our route is also applicable for the synthesis of other members of the bryostatin family (Figure **2.50**). A global deprotection of the bisacetate **2.207** using $LiBF_4$ would provide bryostatin 7. Since the C_{20} acetate can be selectively removed in the presence of the C_7 acetate, other bryostatins having different esters at C_{20} positions can be prepared. For example, esterification of the C_{20} alcohol with propanoic anhydride and subsequent deprotection would provide bryostatin 9. Similarly, Esterification of **2.210** with the C_{20} alcohol followed by global deprotection would furnish bryostatin 15 in which the absolute sterochemistry at the C_{44} alcohol is yet to be determined.[48]

Current route to bryostatin can also be applied in the synthesis of B and C-ring diversified bryostatin analogues (Figure **2.51**). The ketone **2.505** is suitably placed for independent functionalization on the B and the C-ring. Moreover, conversion of C_{20} alcohol to a bulky ester might help the selective removal of the C_7 acetate using

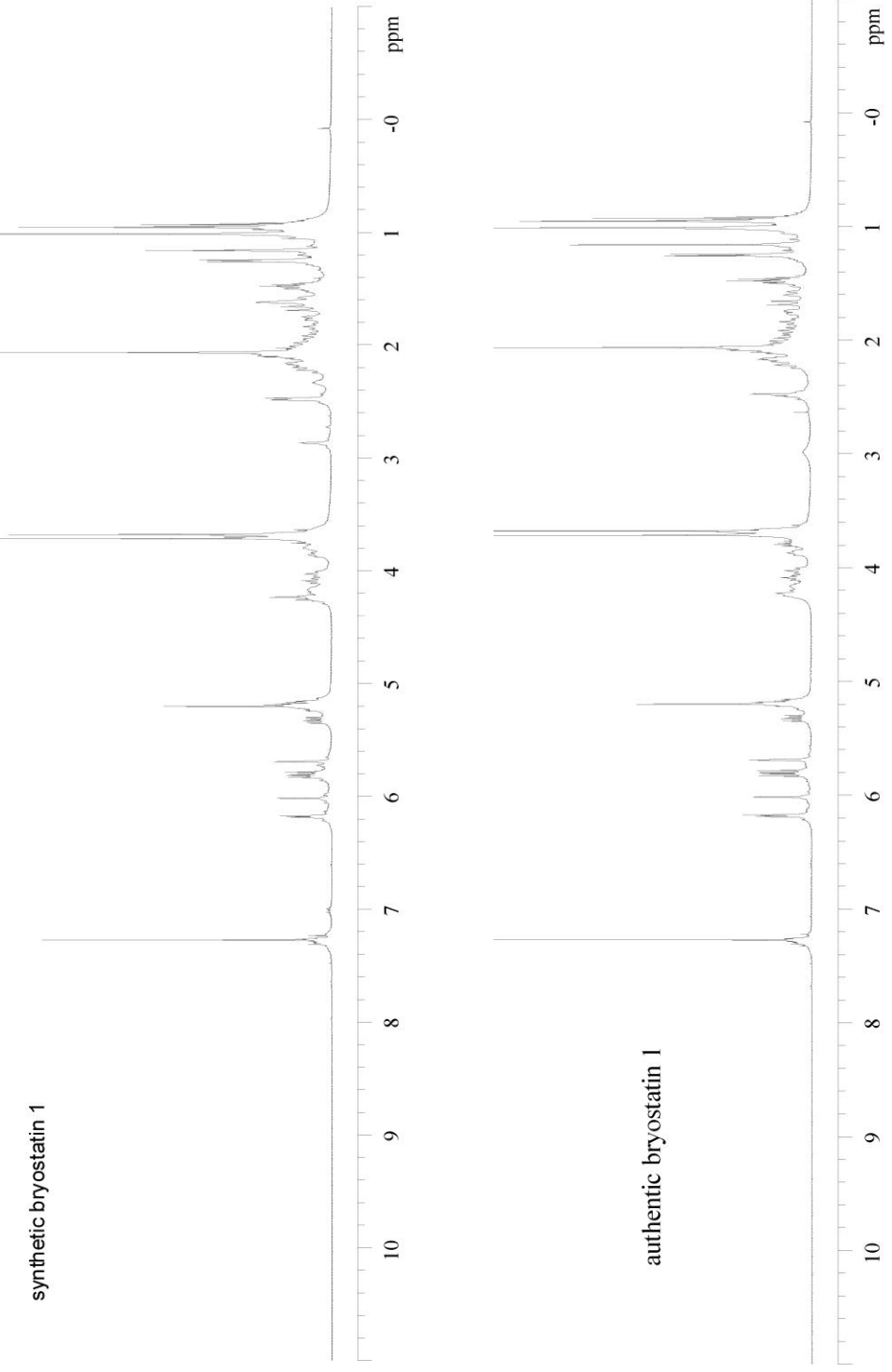


Figure 2.48 ^{1}H NMR of Synthetic and Authentic Bryostatin 1

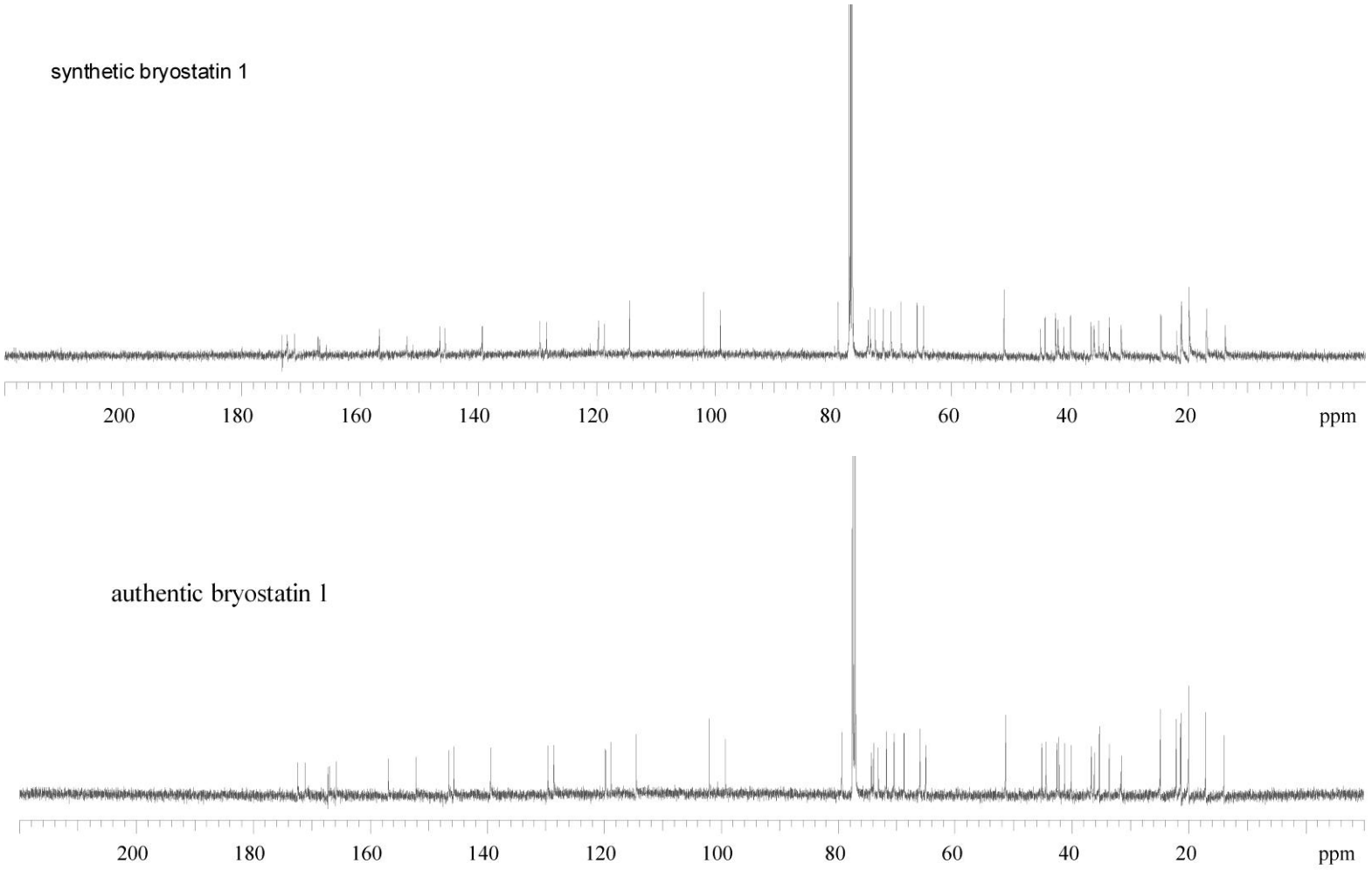


Figure 2.49 ^{13}C NMR of Synthetic and Authentic Bryostatin 1

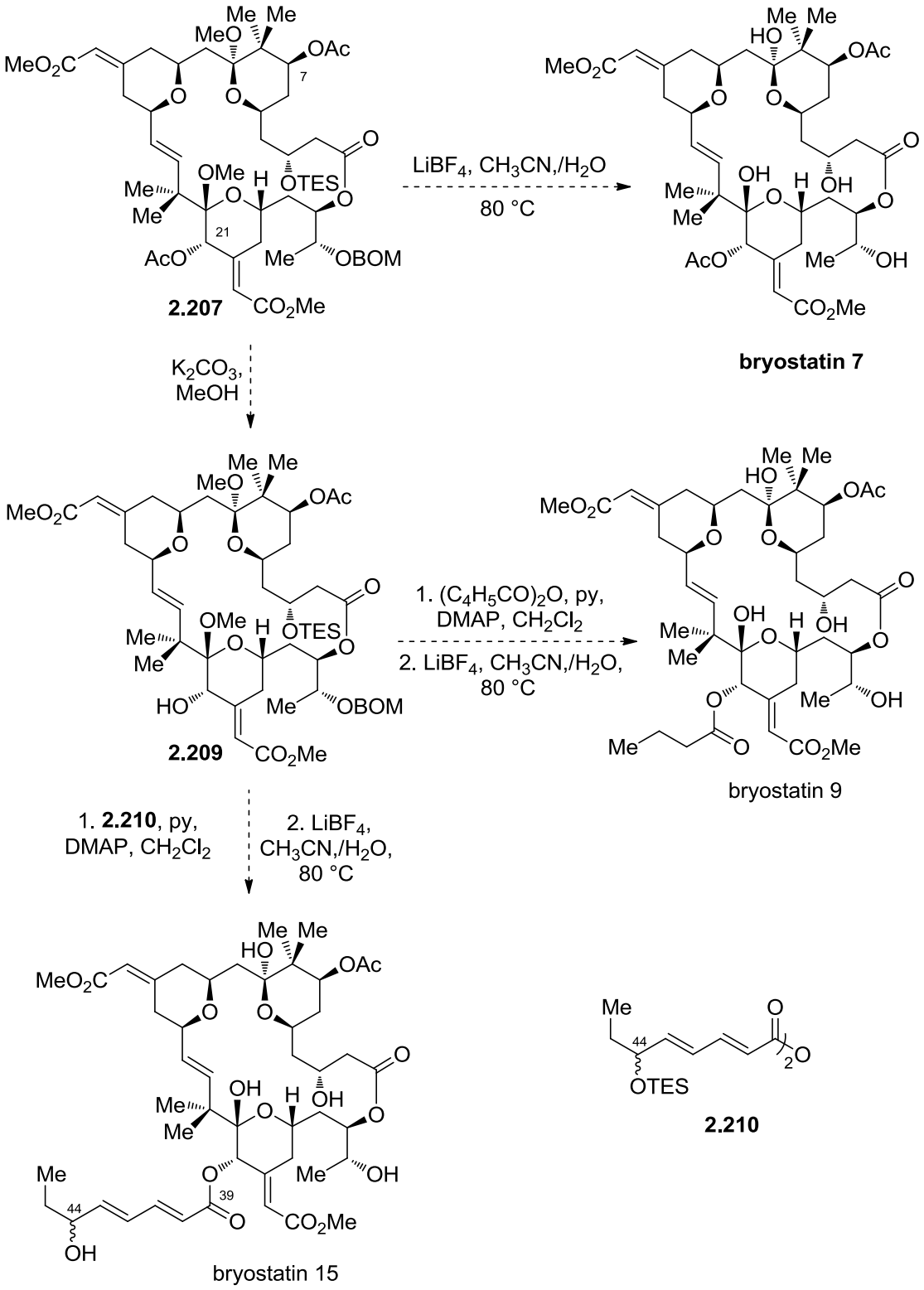


Figure 2.50. Potential Use of Bisacetate 2.207 for the Synthesis of Other Bryostatins

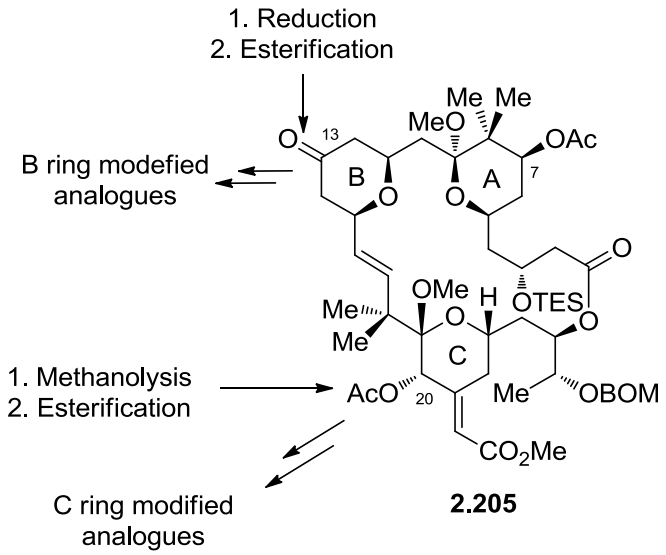


Figure 2.51 Potential Use of Ketone 2.205 in Analogue Synthesis

K_2CO_3/MeOH. If successful, this would lead to the synthesis of A-ring diversified analogues as well.

Conclusions

The first total synthesis of bryostatin 1 was accomplished in 30 steps (longest linear sequence, 55 total steps) from the *R*-isobutyl lactate **2.183**. The preparation of the A-ring hydroxy allylsilane was completed in 17 steps whereas the C-ring aldehyde was made in 18 steps. Thus both of these fragments of similar complexity were prepared in almost the same number of steps. A highly convergent union of these two fragments constructed the macrocycle which after 11 chemical transformations furnished bryostatin 1.

Our synthesis of bryostatin is distinct from other existing syntheses in many

aspects, most notable of these being the assembly of the B-ring pyran of the bryostatin through a highly convergent union of fully functionalized A and C-ring fragments. This represents one of the most complex pyran annulation reactions to date. The gem-dimethyl group on the A-ring was installed through the addition of a novel stannane reagent in a stereoselective fashion. The synthesis is heavily based on stereoselective carbon-carbon bond formation utilizing substrate mediated 1,2 or 1,3 chelation controlled reactions. All 11 sp^3 stereocenters were installed using only two chiral reagents, highlighting the highly substrate controlled nature of the synthesis.

Graphical Summary of Total Syntheses of Bryostatins

A graphical summary of the total syntheses of various bryostatins is shown in Figure **2.52**, **2.53**, **2.54**, **2.56** and **2.57**. Our total synthesis of bryostatin 1 utlizes a convergent coupling of fully functionalized A- and the C-ring to construct the B-ring. On the other hand, Masamune, Evans and Yamamura use the same basic disconnection in which the A-B ring system is connected to the C-ring using Julia coupling. Trost's synthesis of bryostatin 16 is based on the alkane-alkyne coupling to construct the B-and the C-rings.

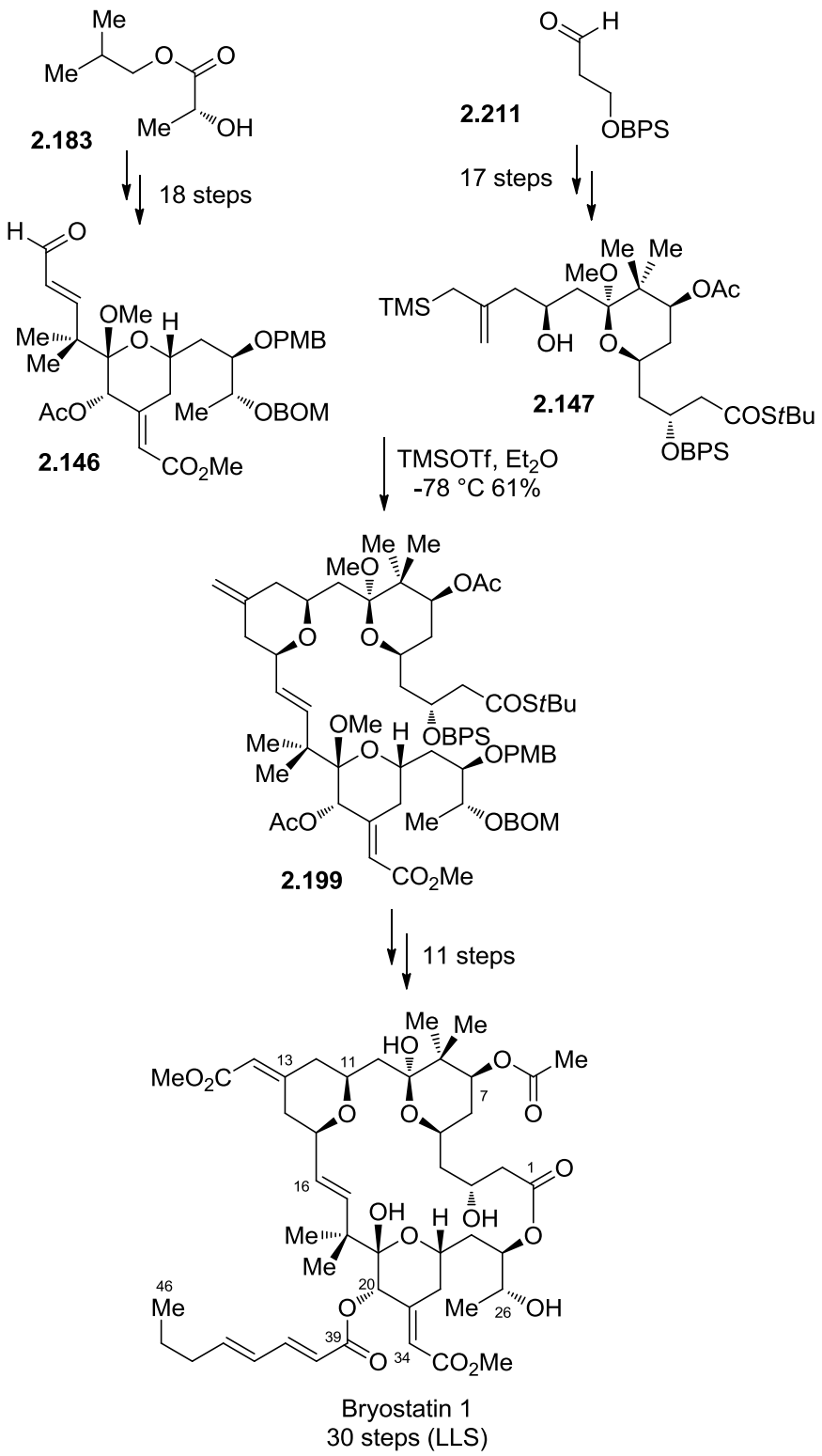


Figure 2.52. Summary of Keck's Total Synthesis of Bryostatin 1

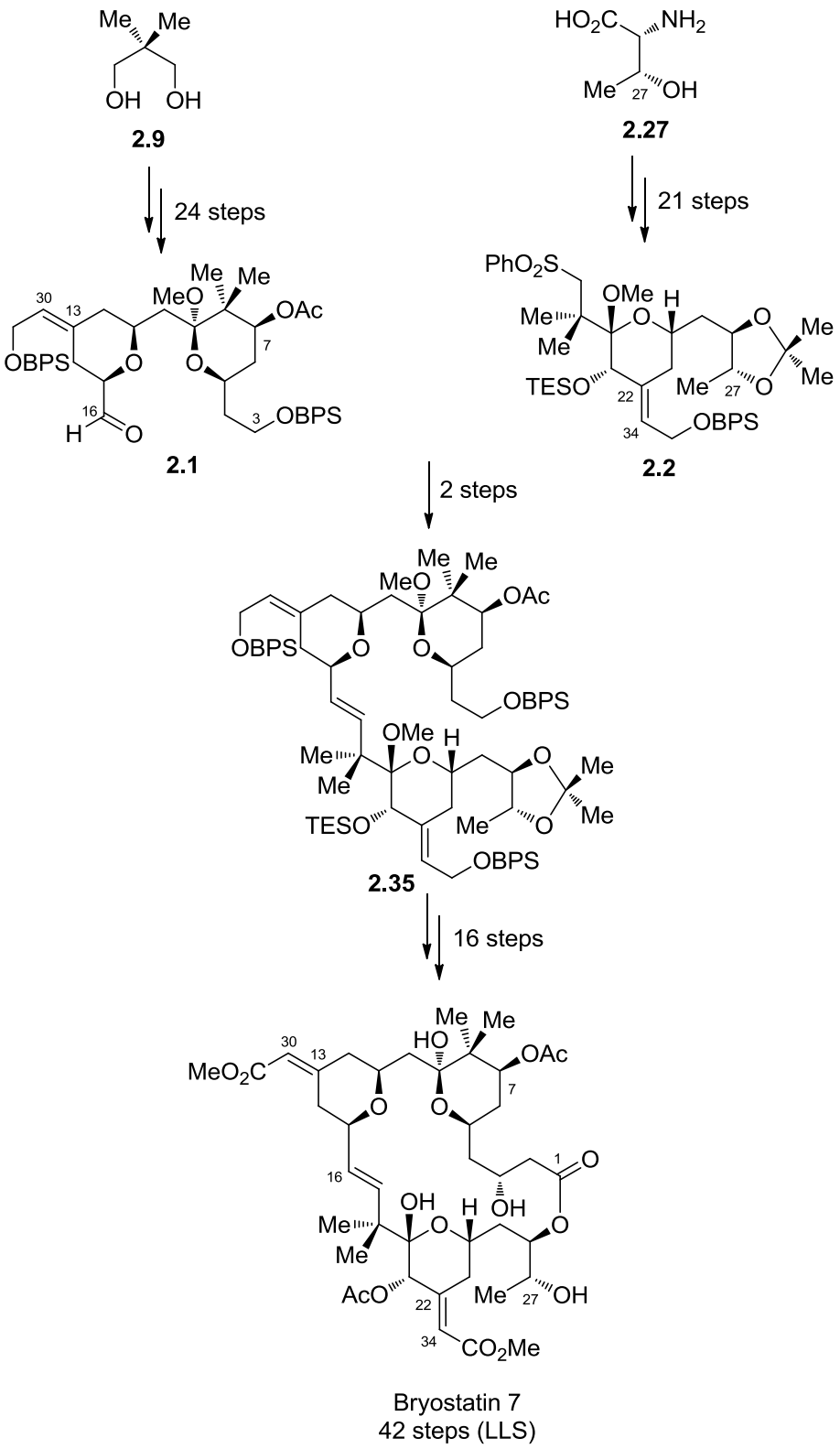


Figure 2.53. Summary of Masamune's Total Synthesis of Bryostatin 7

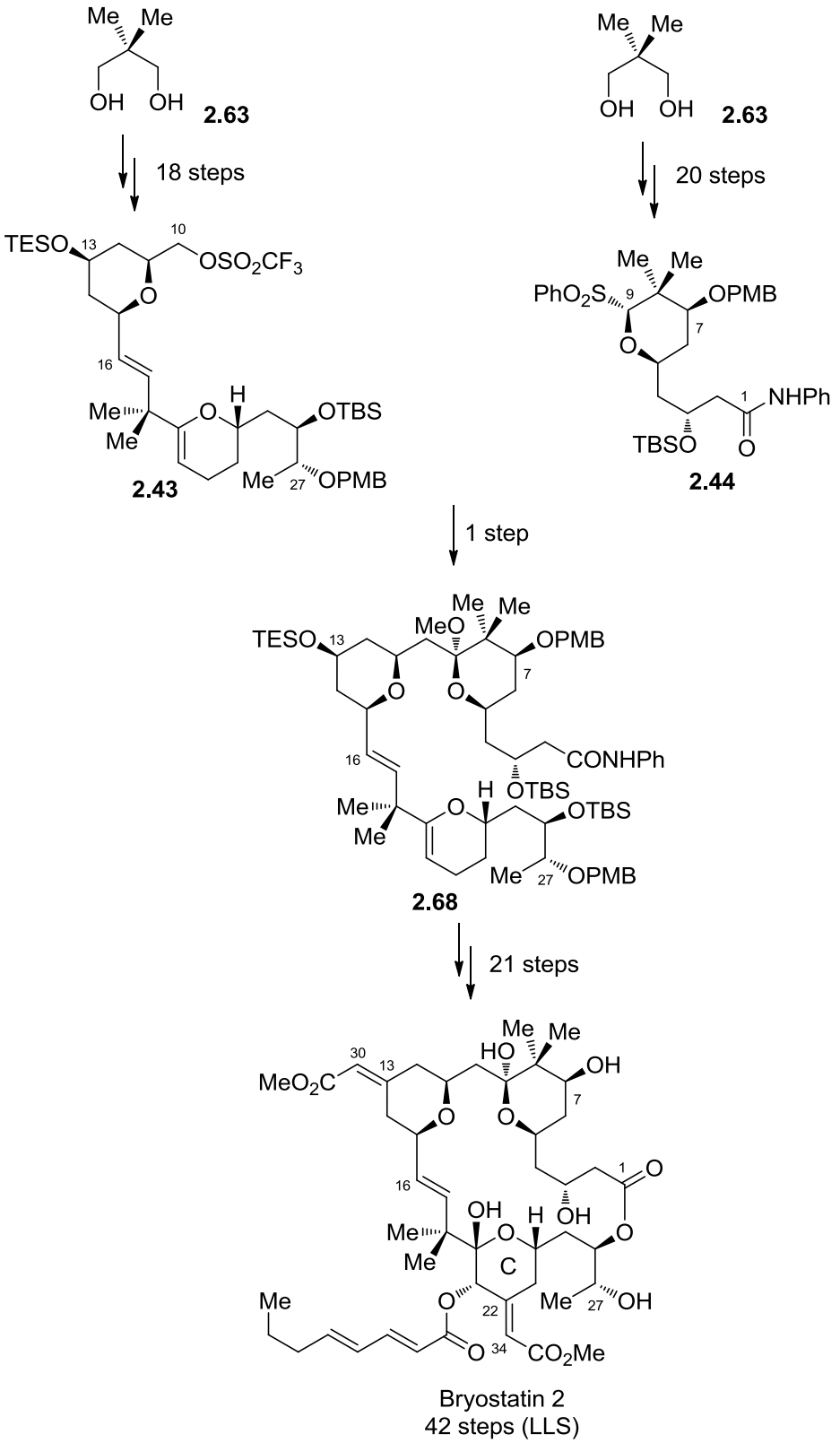


Figure 2.54 Summary of Evans' Synthesis of Bryoststin 2

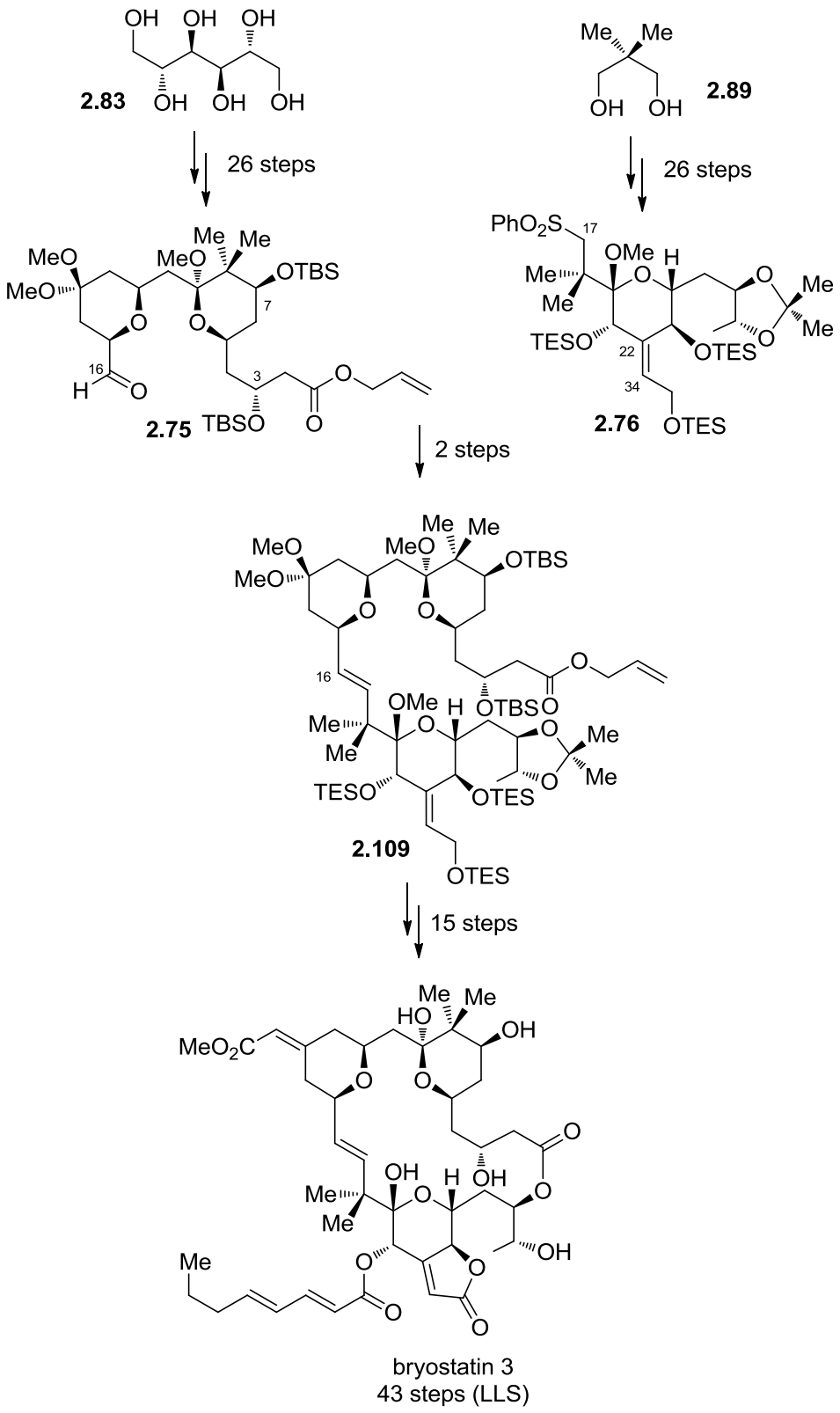


Figure 2.55. Summary of Yamamura's Synthesis of Bryostatin 3

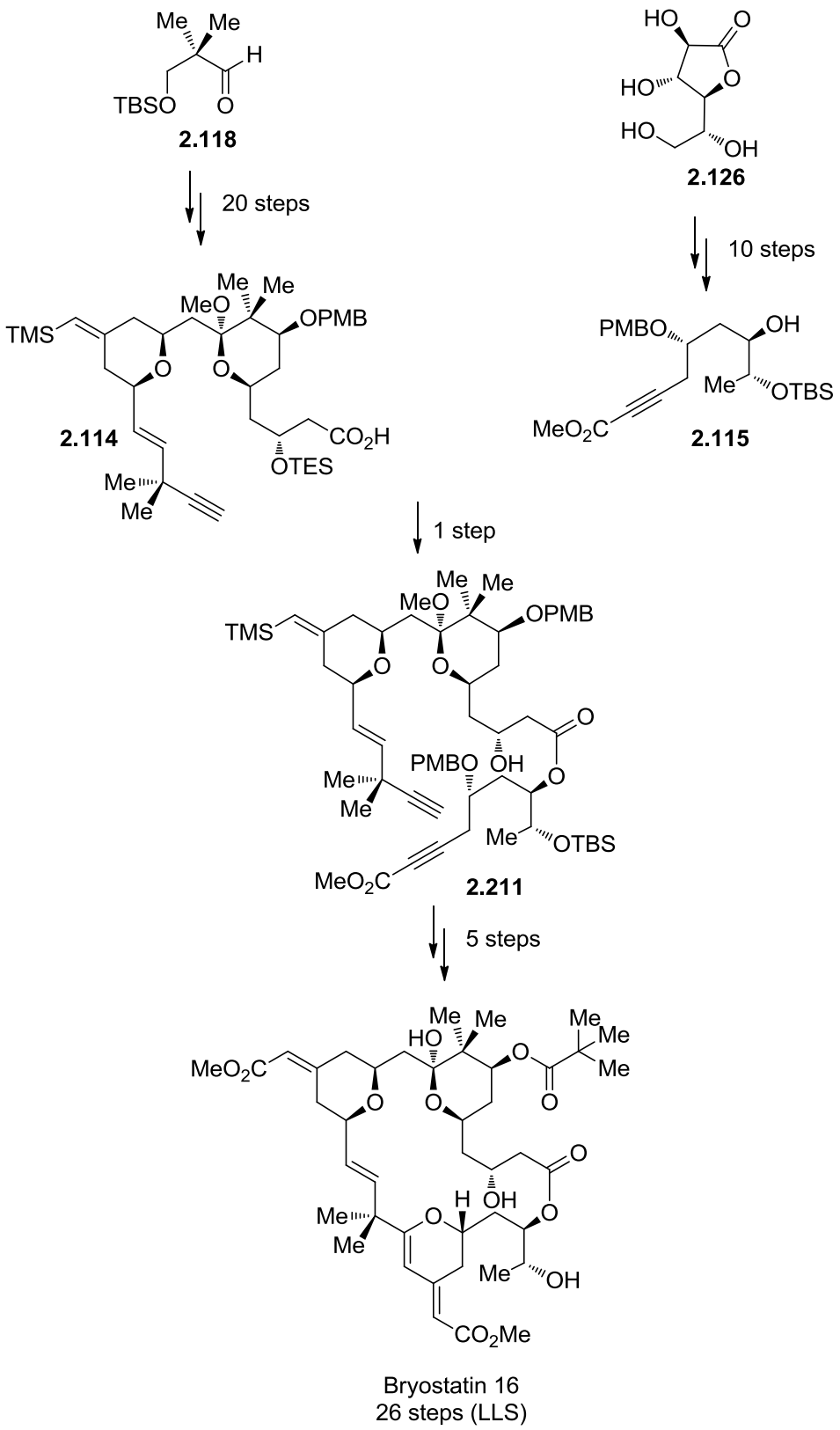


Figure 2.56 Summary of Trost's Synthesis of Bryoststin 16

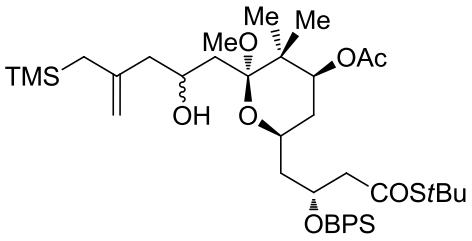
Experimental Section

General Experimental Procedures, Materials and Instrumentation

Solvents were purified according to the guidelines in *Purification of Common Laboratory Chemicals* (Perrin, Armarego, and Perrin, Pergamon: Oxford, 1966).[48] Diisopropylamine, diisopropylethylamine, pyridine, triethylamine, EtOAc, MeOH, and CH_2Cl_2 were distilled from CaH_2. The titer of *n*-BuLi was determined by the method of Eastham and Watson.[49] All other reagents were used without further purification. Yields were calculated for material judged homogenous by thin layer chromatography and nuclear magnetic resonance (NMR). Thin layer chromatography was performed on Merck Kieselgel 60 Å F_{254} plates or Silicycle 60Å F_{254} eluting with the solvent indicated, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 12-molybdophosphoric acid, or 4-anisaldehyde. Flash column chromatography was performed with Silicycle Flash Silica Gel 40 – 63 μm or Silicycle Flash Silica Gel 60 – 200 μm, slurry packed with 1% EtOAc/hexanes in glass columns. Preparative thin layer chromatography was performed on Silicycle 60Å F_{254} 20 cm × 20 cm × 250 μm plates. Glassware for reactions was oven dried at 125 °C and cooled under a dry nitrogen atmosphere prior to use. Liquid reagents and solvents were introduced by oven dried syringes through septum-sealed flasks under a nitrogen atmosphere. Nuclear magnetic resonance spectra were acquired at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts for proton nuclear magnetic resonance (^{1}H NMR) spectra are reported in parts per million relative to the signal of relative to the signal of residual $CHCl_3$ at 7.27 ppm. Chemical shifts for proton nuclear magnetic resonance (^{1}H NMR) spectra are reported in parts per million relative to the signal residual $CDCl_3$ at 7.27 ppm . Chemical shifts for carbon nuclear magnetic resonance (^{13}C NMR and DEPT) spectra are reported in parts

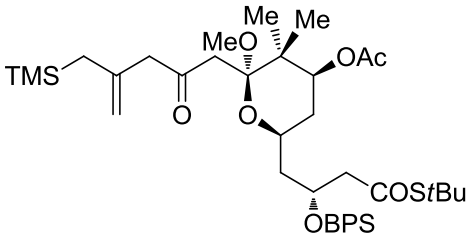
per million relative to the center line of the $CDCl_3$ triplet at 77.23 ppm. Chemical shifts of the unprotonated carbons ('C') for DEPT spectra were obtained by comparison with the ^{13}C NMR spectrum. The abbreviations s, d, apd, dd, ddd, dddd, t, td, tt, q, dq, and m stand for the resonance multiplicity singlet, doublet, apparent doublet, doublet of doublets, doublet of doublet of doublets, doublet of doublet of doublet of doublets, doublet of doublet of doublets of doublets, triplet, triplet of doublets, triplet of triplets, quartet, doublet of quartets, and multiplet, respectively. Optical rotations (Na D line) were obtained using a microcell with 1 dm path length. Specific rotations ($[\alpha]_D^{20}$, Unit: $°cm^2/g$) are based on the equation $\alpha = (100 \cdot \alpha)/(l \cdot c)$ and are reported as unit-less numbers where the concentration *c* is in g/l00 mL and the path length *l* is in decimeters. Mass spectrometry was performed at the mass spectrometry facility of the Department of Chemistry at The University of Utah on a double focusing high resolution mass spectrometer. Compounds were named using using ChemDraw 12.0.

Experimental Procedures and Analytical and Data



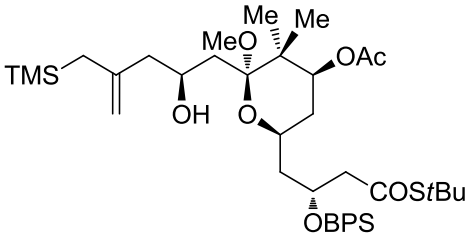
Preparation of ((2*S*,4*S*,6*S*)-6-((*R*)-2-((*tert*-butyldiphenyl silyl)oxy) -4-(*tert*-butylthio)-4-oxobutyl)-2-(2-hydroxy-4-((trimethylsilyl)methyl) pent-4-en-1-yl)-2-methoxy-3,3-dimethyltetrahydro-2H-pyran-4-yl acetate (2.174): To a solution of aldehyde **2.173** (320 mg, 0.48 mmol, 1 equiv) in toluene (1 mL) in a 4 mL vial was added trimethyl(2-tributylstannylmethyl)allylsilane (407 mg, 0.97 mmol, 1 equiv) via syringe. The mixture

was heated at reflux for 12 h at which time TLC analysis showed the reaction was complete. The reaction mixture was allowed to cool to rt and the solvent was removed under reduced pressure. Purification was accomplished by flash column chromatography using a 2 × 10 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (23-66) were combined and concentrated under reduced pressure to provide alcohol **2.174** (325 mg, 85%) as a 1:1 mixture of diastereomers and as colorless oil. R_f = 0.6 (20% EtOAc/hexanes); $[\alpha]_D^{20} =$ +15 (*c* = 1.5, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.65 (m, 4H), 7.44-7.36 (m, 6H), 5.06-5.01 (m, 1H), 4.65-4.61 (m, 2H), 4.28-4.19 (m, 1H), 4.10-3.88 (m, 1H), 3.49-3.44 (m, 1H), 3.13-3.11 (m, 1H), 3.01 (s, 3H), 2.75-2.57 (m, 2H), 2.21-2.13 (m, 1H), 2.02 (s, 3H), 2.00-1.47 (m, 9H), 1.44-1.43 (m, 9H), 1.03 (s, 9H), 0.90-0.82 (m, 6H), 0.04-0.03 (m, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 197.7, 197.6, 173.1, 170.6, 170.5, 144.6, 144.3, 136.0, 135.9, 134.0 (×2), 133.7, 133.6, 129.9 (×3), 127.8 (×3), 110.3 (×2), 105.3, 104.8, 73.3, 73.2, 68.8, 68.5, 67.0, 66.8, 66.3, 66.2, 52.5, 52.2, 49.0, 48.3 (×2), 48.1, 46.6, 46.1, 43.8, 43.6, 42.4, 42.0, 39.5, 37.9, 32.7, 31.7, 29.9 (×2), 27.1, 27.0, 26.9, 21.4, 20.6, 20.4, 19.5 (×2), 17.5, 16.8, -1.12, -1.14; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 51.3, 50.3, 32.2 (×2), 29.4, 23.6, 22.8, 22.6, 19.8, 19.1, -1.14; CH_2 δ 112.6 (×2), 54.7, 54.5, 48.8, 48.4, 46.1, 45.9, 41.8, 40.1, 35.0, 29.3, 29.2, 25.1; CH_0 δ 138.3, 138.2, 132.2, 130.1, 75.6, 75.4, 71.0, 70.8, 69.3, 69.1, 68.6, 68.5; IR (neat) 3071, 2956, 2858, 1742, 1680, 1472, 1427, 1364, 1246, 1111, 1074, 1027, 976, 849, 739, 703 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{43}H_{68}NaO_7SSi_2$ (M+Na): 807.4122, found: 807.4120.



Preparation of (2*S*,4*S*,6*S*)-6-((*R*)-2-((tert-butyldiphenylsilyl)oxy)-4-(*tert*-butylthio)-4-oxobutyl)-2-methoxy-3,3-dimethyl-2-(2-oxo-4-((trimethylsilyl) methyl)pent-4-en-1-yl)tetrahydro-2H-pyran-4-yl acetate (2.175): To a stirring solution of alcohol **2.174** (325 mg, 0.414 mmol**,** 1.0 equiv) in CH_2Cl_2 (4 mL) in a 15 mL rb flask at -15 °C, was added freshly distilled *N*,*N*-diisopropylethylamine (504 µL, 2.8 mmol, 7.0 equiv), dropwise via syringe. After 10 min at -15 °C, dimethyl sulfoxide (293 µL, 4.1 mmol, 10.0 equiv) was added to the reaction mixture via syringe and the solution was allowed to stir for an additional 10 min. Sulfur trioxide pyridine complex (263 mg, 1.6 mmol, 4.0 equiv) was then added in one portion. The reaction was allowed to proceed for 1 h at -15 °C, after which time TLC analysis indicated complete consumption of starting material. The reaction mixture was diluted with EtOAc (10 mL) and quenched by the addition of saturated aqueous $NaHCO_3$ solution (10 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine (2 × 10 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 2 × 8 cm column, eluting with 5% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (16-26) were combined and concentrated under reduced pressure to give the ketone **2.175** (301 mg, 93% yield) as a white foam. R_f = 0.37 (10% EtOAc/hexanes); $[\alpha]_D^{20} =$ +8 (*c* = 1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.69-7.65 (m, 4H), 7.45-7.36 (m, 6H), 4.99 (dd, *J*

= 11.7, 5.1 Hz, 1H), 4.70 (s, 1H), 4.67 (s, 1H), 4.23 (q, *J* = 6.4 Hz, 1H), 3.55 (dddd, *J* = 9.9, 6.7, 6.7, 3.3 Hz, 1H), 3.15-3.07(m, 3H), 3.07 (s, 3H), 2.77 (d, *J* = 13.5, 2H), 2.68 (dd, *J* = 14.6, 6.5 Hz, 1H), 2.61 (dd, *J* = 14.2, 5.1 Hz, 1H), 2.14 (d, *J* = 16.9 Hz, 2H), 2.01 (s, 3H), 1.79-1.73 (m, 1H), 1.52 (d, *J* = 2.5 Hz, 2H), 1.44 (s, 9H), 1.03 (s, 9H), 0.94 (s, 3H), 0.80 (s, 3H), 0.03 (s, 9H) ; 125 MHz ^{13}C NMR ($CDCl_3$) δ 206.1, 197.6, 170.5, 141.2, 136.1, 135.9, 134.2, 133.6, 129.9, 129.8, 127.8(×2), 112.3, 104.4, 73.1, 68.8, 66.2, 54.3, 52.6, 49.2, 48.2, 44.3, 43.6, 42.5, 32.6, 29.9, 27.1, 26.6, 21.3, 20.6, 19.5, 17.4, -1.1 ; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 49.2, 29.9, 27.1, 21.4, 20.6, 17.4, -1.1; CH_2 δ 112.3, 54.3, 52.6, 44.3, 43.6, 32.6, 26.6; CH_1 δ 136.1, 136.0, 130.0, 129.9, 127.8 (×2), 73.1, 68.8, 66.2,; CH_0 δ 206.1, 197.6, 170.5, 141.2, 134.2, 133.6, 129.8, 104.4, 73.1, 48.2, 42.5, 19.5; IR (neat) 3049, 2956, 2858, 1740, 1679, 1631, 1472, 1427, 1389, 1365, 1246, 1111, 1074, 1028, 996, 849, 759, 703, 611 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{43}H_{66}NaO_7SSi_2$ (M+Na): 805.3966, found: 805.3965.



Preparation of (2*S*,4*S*,6*S*)-6-((*R*)-2-((*tert*-butyl diphenylsilyl)oxy)-4-(*tert*-butylthio) -4-oxobutyl)-2-((*S*)-2-hydroxy -4-((trimethylsilyl) methyl) pent-4-en-1-yl)-2-methoxy-3,3-dimethyltetrahydro-2H-pyran-4-yl acetate (2.147): To a stirring solution of ketone **2.175** (206 mg, 0.263 mmol, 1.0 equiv) in MeOH (26 mL, 0.01 M) in a 50 mL rb flask at rt was added $CeCl_3 \cdot 7H_2O$ (1.9 g, 5.3 mmol, 20 equiv). The mixture was stirred until all the $CeCl_3 \cdot 7H_2O$ was completely dissolved. The mixture was then cooled to -42 ºC and stirred for 10 min, and then $NaBH_4$ (100 mg, 2.63 mmol, 10.0 equiv) was added. Stirring continued for 5 h at

-42 ºC after which the reaction was warmed to 0 ºC and stirred for an additional 1 h, then quenched by the slow addition of saturated aqueous NH_4Cl solution (5 mL) and diluted with 40% EtOAc/hexanes (10 mL). The layers were separated and the aqueous layer was extracted with 40% EtOAc/hexanes (2 × 10 mL). The organic phase was washed with brine (5 mL), then dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 2 × 7 cm column, eluting with 5% EtOAc/hexanes, collecting 4 mL fractions. The products containing fractions (21-48) were combined and concentrated under reduced pressure to give the alcohol **2.147** (171 mg, 82% yield) as a 4:1 mixture of diastereomers as measured by NMR. For analytical purpose, a small portion of the mixture of diastereomers was further purified using preparative thin layer chromatography eluting with 1% EtOAc in benzene to give the pure desired major isomer as a colorless oil: R_f = 0.3 (10% EtOAc/hexanes); $[\alpha]_D^{20} =$ +16.7 (*c* = 1.1, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.64 (m, 4H), 7.45-7.36 (m, 6H), 5.04 (dd, *J* = 11.7, 4.8 Hz, 1H), 4.66-4.61 (m, 2H), 4.25 (q, *J* = 5.8 Hz, 1H), 4.07 (q, *J* = 7.3 Hz, 1H), 3.46 (dddd, *J* = 10.3, 8.3, 5.4, 3.5 Hz, 1H), 3.13(s, 1H), 3.01 (s, 3H), 2.73 (dd, *J* = 14.6, 6.3, 1H), 2.63 (dd, *J* = 14.6, 6.3 Hz, 1H), 2.18 (dd, *J* = 13.6, 5.8 Hz, 1H), 2.02 (s, 3H), 1.94 (dd, *J* = 13.1, 6.8 Hz, 1H), 1.81-1.76 (m, 4H), 1.54 (d, *J* = 1.4 Hz, 2H), 1.44 (s, 9H), 1.03 (s, 9H), 0.90 (s, 3H), 0.82 (s, 3H), 0.04 (s, 9H) ; 125 MHz ^{13}C NMR ($CDCl_3$) δ 197.0, 170.6, 144.4, 136.1, 136.0, 134.0, 133.7, 130.0, 129.9, 127.8(×2), 110.3, 104.8, 73.2, 68.8, 67.1, 66.3, 52.5, 49.0, 48.3, 46.1, 43.8, 42.0, 39.5, 32.8, 29.9, 27.1, 21.4, 20.4, 19.5, 17.6, -1.1; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 49.0, 29.9, 27.1, 21.4, 20.4, 17.6, -1.1; CH_2 δ 110.3, 52.5, 46.1, 43.8, 39.5, 32.8, 29.9; CH_1 δ 136.1, 136.0, 130.0(×2), 127.9(×2), 73.2, 68.8, 67.1, 66.3; CH_0 δ 197.8,

170.6, 144.4, 134.0, 133.7, 104.8, 48.3, 42.0, 19.5; IR (neat) 2956, 2858, 1742, 1680, 1427, 1366, 1246, 1111, 1074, 1027, 849, 703 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{43}H_{68}NaO_7SSi_2$ (M+Na): 807.4122, found: 807.4120.

Verification of stereochemistry at the C_{11} position of the compound 2.147: In order to verify the stereochemistry of the C_{11} alcohol, a pyran annulation was carried out between the silane **2.147** and hydrocinnamaldehyde using TMSOTf to furnish the compound **2.182** (Figure **2.57**). On the other hand, an authentic compound **2.180** with known stereochemistry at C_{11} was prepared independently using a different pyran annulation between the aldehyde **2.173** and the known silane **2.179**. Since the pyran annulation takes place with retention of configuration at the alcohol center and the NMR spectra of the compounds **2.180** and **2.182** were identical in different solvents such as

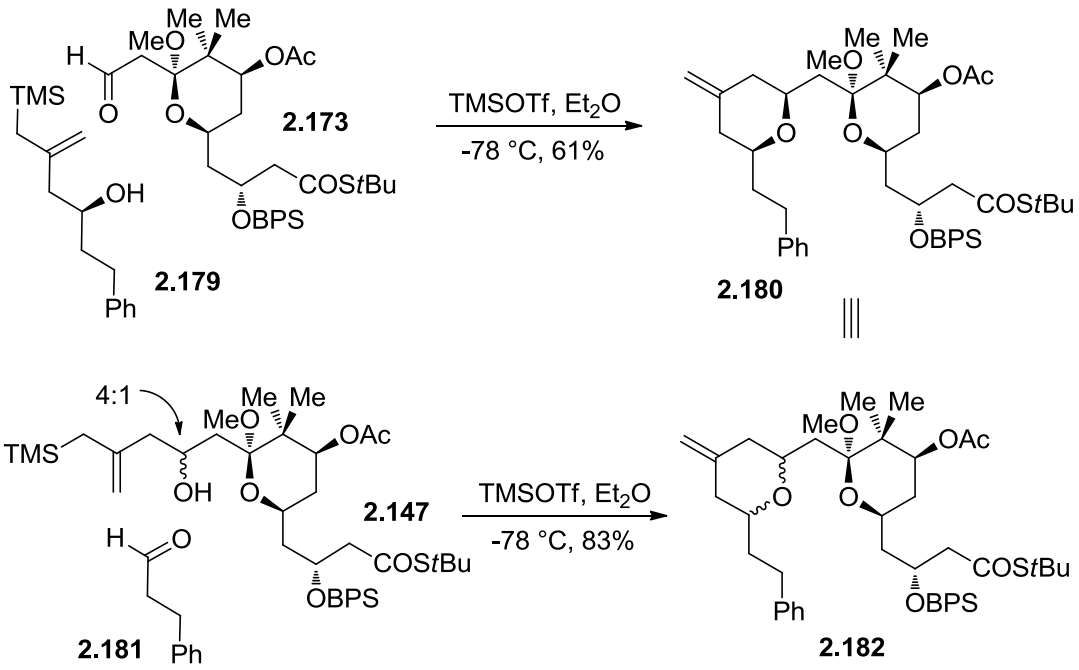
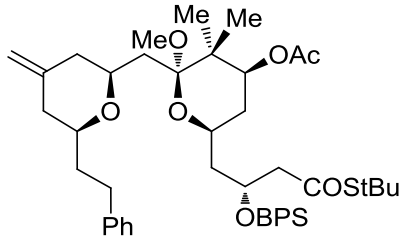


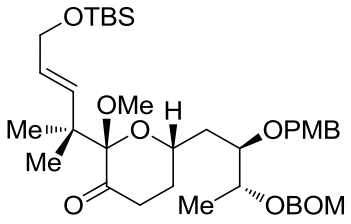
Figure 2.57 Verification of the C_{11} Stereochemistry

$CDCl_3$ and C_6D_6. This verifies that the hydroxyl group at the C_{11} position of the major isomer of compound **2.147** has the desired configuration.



Preparation of (2*S*,4*S*,6*S*)-6-((*R*)-2-((*tert*-butyl diphenylsilyl)oxy)-4-(*tert*-butylthio)-4-oxobutyl)-2-methoxy-3,3-dimethyl-2-(((2*S*,6*S*)-4-methylene-6-phenethyltetrahydro-2H-pyran-2-yl)methyl)tetrahydro-2H-pyran-4-yl acetate (2.180): To a stirring solution of aldehyde **2.173** (21 mg, 0.032 mmol, 1.0 equiv) and hydroxyallylsilane **2.179** (8 mg, 0.032 mmol, 1.0 equiv) in Et_2O (460 µL) in a flame dried 5 mL vial at -78 ºC was added a solution of TMSOTf in Et_2O (38 µL of 1.0 M,, 0.038 mmol, 1.2 equiv) via syringe. After 3 h at -78 ºC, the reaction mixture was quenched by the addition of *N*,*N*-diisopropylethylamine, followed by the addition of saturated aqueous $NaHCO_3$ solution. The mixture was warmed to rt, the phases were separated, and the aqueous phase was extracted twice with Et_2O. The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. Purification was accomplished by flash column chromatography eluting with hexanes/EtOAc (7:3) to provide the pyran **2.180** (16 mg, 61%) as a white foam. R_f = 0.4 (10% EtOAc/hexanes); $[\alpha]_D^{20} =$ +18 (*c* = 1.1, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.65 (m, 4H), 7.44-7.18 (m, 11H), 4.97 (dd, *J* = 11.7, 4.8 Hz, 1H), 4.68 (dd, *J* = 7.8, 1.9 Hz, 2H), 4.26 (sep, *J* = 3.9 Hz, 1H), 3.42 (dddd, *J* = 7.4, 5.4, 3.4, 1.9 Hz, 1H), 3.21-3.17 (m, 1H), 2.98(s, 3H), 2.85 (ddd, *J* = 14.1, 10.7, 5.3 Hz, 1H), 2.76 (dd, *J* = 14.6, 4.3 Hz, 1H), 2.71 (dd, *J* = 14.6, 7.3 Hz, 1H), 2.58 (ddd, *J* = 13.6, 10.2, 6.3 Hz, 1H), 2.23 (d, *J* = 13.1 Hz, 1H), 2.15 (d, *J* = 13.1 Hz, 1H), 2.01 (s, 3H), 1.99-1.86 (m, 3H), 1.84-1.64 (m, 5H), 1.45 (s, 9H),

1.43 (d, *J* = 1.9 Hz, 1H), 1.32-1.26 (m, 2H), 1.02 (s, 9H), 0.93 (s, 3H), 0.87 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 198.1, 170.7, 145.0, 142.6, 136.1, 136.0, 134.4, 133.6, 129.9, 129.8, 128.6, 128.5, 127.8, 127.7, 125.8, 108.6, 104.0, 77.5, 74.6, 73.9, 69.5, 66.0, 53.2, 48.2(×2), 43.7, 42.4, 41.9, 40.8, 39.2, 38.6, 32.8, 32.2, 30.0, 27.1, 21.4, 20.8, 19.5, 16.9; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 48.2, 30.0, 27.1, 21.4, 20.8, 16.9; CH_2 δ 108.6, 53.2, 43.7, 42.4, 40.8, 39.2, 38.6, 32.8, 32.2; CH_1 δ 136.1, 136.0, 129.9, 129.8, 128.6, 128.5, 127.8, 127.7, 125.9, 77.5, 74.6, 73.9, 69.5, 66.0; CH_0 δ 198.1, 170.7, 145.0, 142.6, 134.6, 133.6, 104.0, 48.2, 41.9, 19.5; IR (neat) 3050, 2932, 2858, 1742, 1682, 1455, 1427, 1364, 1243, 1110, 890, 822, 739, 701, 611cm^{-1}; HRMS (ESI/APCI) calcd for $C_{49}H_{68}NaO_7SSi$ (M+Na): 851.4353, found: 851.4362.

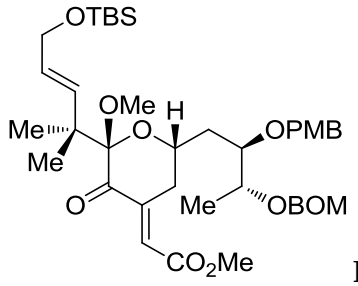


Preparation of (2*S*,6*S*)-6-((2*R*,3*R*)-3-((benzyloxy) methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-((*E*)-5-((tert-butyldimethylsilyl)oxy)-2-methylpent-3-en-2-yl)-2-methoxydihydro-2H-pyran-3(4H)-one (2.156): To a stirring solution of dihydropyran **2.157** (100 mg, 0.16 mmol, 1.0 equiv) in CH_2Cl_2 (1.6 mL) at 0 °C was added MeOH (0.8 mL). Powdered $NaHCO_3$ (34 mg, 0.4 mmol, 2.5 equiv) was added in one portion and the solution was stirred at 0 °C for 10 min. Magnesium monoperoxyphthalate (80%, 197 mg, 0.32 mmol, 2.0 equiv) was added slowly and the mixture was stirred for 1 h at 0 °C. The reaction mixture was then quenched by the addition of saturated aqueous $NaHCO_3$ solution (5 mL), diluted with EtOAc (5 ml) and the layers were separated. The aqueous layer was extracted with EtOAc (3 × 5 mL). The

combined organic layers were washed with water (5 mL), and with brine (5 mL), then dried over Na_2SO_4, filtered, concentrated, and taken into the next step without further purification.

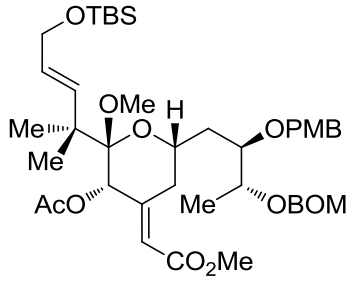
To this intermediate alcohol in CH_2Cl_2 (3 mL), at rt, were added 4 Å molecular sieves (200 mg), TPAP (6 mg, 0.016 mmol, 0.1 equiv), and 4-methylmorpholine-N-oxide (56 mg, 0.48 mmol, 3.0 equiv). The mixture was stirred at rt for 1 h and then diluted with EtOAc (10 mL). The mixture was then filtered through a small plug of Florisil® and washed with copious amounts of EtOAc. The solvent was removed under reduced pressure and purification was accomplished by flash column chromatography, using a 2 × 7 cm silica gel column, eluting with 15% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (14-27) were combined and concentrated under reduced pressure to provide methoxyketone **2.156** (74 mg, 68% over 2 steps) as a colorless oil. R_f = 0.4 (10% EtOAc/hexanes); $[\alpha]_D^{20} =$ +10.3 (*c* = 1.1, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.20 (m, 7H), 6.85-6.83 (m, 2H), 5.97 (dt, *J* = 15.6, 1.9 Hz, 1H), 5.49 (dt, *J* = 16.1, 4.8 Hz, 1H), 4.85 (d, *J* = 6.8 Hz, 1H), 4.83 (d, *J* = 7.3 Hz, 1H), 4.66 (s, 2H), 4.62 (d, *J* = 11.2 Hz, 1H), 4.44 (d, *J* = 10.7 Hz, 1H), 4.14 (dd, *J* = 5.3, 1.4 Hz, 2H), 4.12-4.06 (m, 2H), 3.87 (ddd, *J* = 10.2, 4.8, 1.9 Hz, 1H), 3.79 (s, 3H), 3.22 (s, 3H), 2.42 (dd, *J* = 8.5, 5.6 Hz, 2H), 1.99-1.85 (m, 3H), 1.65 (ddd, *J* = 13.1, 10.2, 2.9 Hz, 1H), 1.21 (d, *J* = 6.3 Hz, 3H), 1.13 (s, 3H), 1.09 (s, 3H), 0.90 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 207.6, 159.3, 138.0, 136.1, 130.7, 129.4, 128.6, 128.0, 127.9, 127.8, 114.0, 104.2, 93.5, 77.3, 72.6, 72.1, 70.1, 69.6, 64.2, 55.4, 52.3, 44.1, 37.7, 36.4, 30.2, 26.1, 22.9, 22.1, 18.5, 14.9, -4.9; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 55.4, 52.3, 26.1, 22.9, 22.1, 14.9, -4.9; CH_2 δ 93.5, 72.1, 69.6, 64.2, 37.7, 36.4, 30.2; CH δ 136.1,

129.4, 128.6, 127.9, 127.8, 127.7, 113.9, 77.3, 72.6, 70.1; CH_0 δ 207.6, 159.3, 138.0, 130.7, 104.2, 44.1, 18.5. IR (thin film) 2953, 2856, 1723, 1612, 1513, 1462, 1382, 1249, 1112, 1042, 836, 777, 737, 698 cm^{-1}; HRMS (ESI) calcd for $C_{38}H_{58}NaO_8Si$ (M+Na) 693.3799, found 693.3802.



Preparation of (*E*)-methyl 2-((2*S*,6*S*)-6-((2*R*,3*R*)-3-((benzyloxy) methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-((*E*)-5-((*tert*-butyldimethyl silyl)oxy)-2-methylpent-3-en-2-yl)-2-methoxy-3-oxodihydro-2H-pyran-4(3H)-ylidene) acetate (2.193): To a stirring solution of ketone **2.157**(237 mg, 0.35 mmol, 1.0 equiv) in MeOH (3.5 mL) at rt were added K_2CO_3 (243 mg, 1.76 mmol, 5 equiv) and a solution of freshly distilled methyl glyoxylate in THF (590 μL of 3M, 1.76 mmol, 5 equiv). The mixture was stirred for 1 h, during which time the color of the solution changed to yellow, then quenched by pouring into saturated aqueous NH_4Cl solution (10 mL). Ether (10 mL) was added, the phases were separated, and the aqueous phase was extracted with Et_2O (3 × 10 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification was accomplished by flash column chromatography, using a 2 × 7 cm silica gel column, eluting with 15% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (6-18) were combined and concentrated under reduced pressure to provide enoate **2.193** (210 mg, 80%) as a bright yellow oil. R_f = 0.3 (10% Et_2O/40% hexanes/50% CH_2Cl_2); $[\alpha]_D^{20}$ = -24.1 (*c* = 1.2, $CHCl_3$); 500 MHz ^1H NMR ($CDCl_3$) δ 7.37-7.16 (m, 7H), 6.83-6.80 (m,

2H), 6.53 (dd, *J* = 2.9, 1.4 Hz, 1H), 5.80 (td, *J* = 16.1, 1.4 Hz, 1H), 5.40 (td, *J* = 16.1, 4.8 Hz, 1H), 4.85 (d, *J* = 6.8 Hz, 1H), 4.83 (d, *J* = 6.3 Hz, 1H), 4.66 (s, 2H), 4.60 (d, *J* = 10.7 Hz, 1H), 4.40 (d, *J* = 11.2 Hz, 1H), 4.15-4.02 (m, 4H), 3.88 (ddd, *J* = 11.7, 3.9, 1.4 Hz, 1H), 3.77 (s, 3H), 3.75 (s, 3H), 3.30 (td, *J* = 17.1, 1.9 Hz, 1H), 3.20 (s, 3H), 2.84 (ddd, *J* = 19.0, 12.6, 3.4 Hz, 1H), 1.95 (ddd, *J* = 14.6, 9.2, 1.9 Hz, 1H), 1.74 (ddd, *J* = 12.6, 9.7, 2.4 Hz, 1H), 1.20 (d, *J* = 6.3 Hz, 3H), 1.10 (s, 3H), 1.04 (s, 3H), 0.89 (s, 9H), 0.04 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 197.7, 166.1, 159.3, 148.2, 138.0, 134.7, 130.6, 129.2, 128.7, 128.6, 128.0, 127.8, 122.6, 113.9, 104.7, 93.5, 76.9, 72.3, 71.7, 69.6, 69.5, 64.1, 55.3, 52.2, 51.8, 44.6, 36.1, 36.0, 26.1, 22.5, 22.0, 18.5, 14.6, -5.0; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 55.3, 52.2, 51.8, 26.1, 22.5, 21.9, 14.6, -5.0; CH_2 δ 93.5, 71.7, 69.6, 64.0, 36.1, 36.0; CH δ 134.7, 129.2, 128.7, 128.6, 128.0, 127.8,122.6, 113.9, 76.9, 72.3, 69.5; CH_0 δ 197.7, 166.1, 159.3, 148.2, 138.0, 130.6, 104.7, 44.6, 18.5. IR (thin film) 2933, 2886, 2856, 1724, 1612, 1513, 1462, 1383, 1178, 835, 777, 739, 698 cm^{-1}; HRMS (ESI) calcd for $C_{41}H_{60}NaO_{10}Si$ (M+Na) 763.3853, found 763.3856.

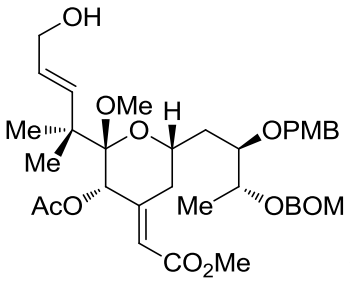


Preparation of (*E*)-methyl 2-((2*S*,3*S*,6*S*)-3-acetoxy-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-((*E*)-5-((*tert*-butyldime thylsilyl)oxy)-2-methylpent-3-en-2-yl)-2-methoxydihydro-2H-pyran-4(3H)-ylidene) acetate (2.194): To a stirring solution of ketone **2.103** (554 mg, 0.74 mmol, 1.0 equiv) in MeOH (74 mL, 0.01 M) at rt was added $CeCl_3 \cdot 7H_2O$ (5.6 g, 14.9 mmol, 20.0 equiv). The resulting mixture was stirred until all the $CeCl_3 \cdot 7H_2O$ was

completely dissolved. The mixture was then cooled to -42 ºC and stirred for 10 min, then $NaBH_4$ (283 mg, 7.47 mmol, 10.0 equiv) was added. Stirring was continued for 1 h at -42 ºC after which the reaction was quenched by the slow addition of saturated aqueous NH_4Cl solution (20 mL) and diluted with 40% EtOAc/hexanes (30 mL). The layers were separated and the aqueous layer was extracted with 40% EtOAc/hexanes (3 × 30 mL). The organic phase was washed with water (20 mL), and with brine (2 × 20 mL), then dried over Na_2SO_4, filtered and concentrated under reduced pressure to provide the crude intermediate alcohol. The alcohol was found to be unstable for purification, and was thus carried directly to the next step without further purification.

To a stirring solution of this alcohol in CH_2Cl_2 (37 mL, 0.02 M) at 0 ºC were added pyridine (1.2 mL, 14.9 mmol, 20 equiv), DMAP (91 mg, 0.74 mmol, 1 equiv), and acetic anhydride (353 µL, 3.73 mmol, 5 equiv). The reaction mixture was stirred at 0 ºC for 3 h and then quenched by the addition of saturated aqueous $NaHCO_3$ solution (20 mL). The mixture was stirred vigorously for 10 min and was then diluted with 40% EtOAc/hexanes (50 mL). The phases were separated and the aqueous phase was extracted with 40% EtOAc/hexanes (2 × 20 mL). The combined organic phases were washed with brine (2 × 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography using a 3 × 8 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 6 mL fractions. Fractions 21-70 provided the desired product **2.194** as a single diastereomer (495 mg, 84% over 2 steps) and as a colorless liquid. R_f = 0.46 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = -0.8 (*c* = 1.1, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.19 (m, 7H), 6.85-6.82 (m, 2H), 6.00 (dt, *J* = 15.6, 1.4 Hz, 1H), 5.88 (bs, 1H), 5.36 (dt, *J* = 16.1, 5.3 Hz, 1H), 5.37 (s, 1H), 4.87 (d, *J*

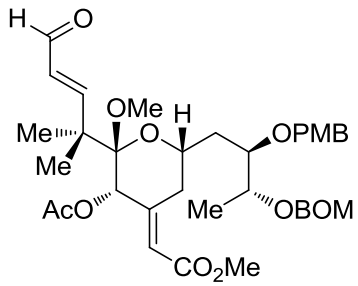
= 7.3 Hz, 1H), 4.84 (d, *J* = 7.3 Hz, 1H), 4.66 (d, *J* = 1.9 Hz, 2H), 4.61 (d, *J* = 10.7 Hz, 1H), 4.42 (d, *J* = 10.7 Hz, 1H), 4.14-4.09 (m, 2H), 4.03 (dddd, *J* = 12.4, 9.2, 2.7, 2.7 Hz, 1H), 3.89 (ddd, *J* = 9.7, 4.3, 1.9 Hz, 1H), 3.78 (s, 3H), 3.68 (s, 3H), 3.51 (dd, *J* = 15.6, 8.5 Hz, 1H), 3.23 (s, 3H), 2.29 (ddd, *J* = 14.6, 11.4, 1.9 Hz, 1H), 2.03 (s, 3H), 1.92 (ddd, *J* = 14.6, 10.2, 2.4 Hz, 1H), 1.73 (ddd, *J* = 14.6, 10.2, 2.4 Hz, 1H), 1.22 (d, *J* = 6.3 Hz, 3H), 1.10 (s, 6H), 0.89 (s, 9H), 0.05 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.0, 166.2, 159.0, 152.2, 138.0, 137.8, 130.3, 129.2, 128.3, 127.6, 127.5, 124.0, 117.3, 113.6, 102.5, 93.1, 76.6, 72.1, 71.8, 71.7, 69.3, 68.3, 64.3, 55.0, 51.4, 50.9, 45.6, 36.2, 32.3, 25.1, 24.6, 23.1, 21.1, 18.3, 14.5, -5.1 (×2); 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 55.0, 51.4, 51.0, 25.9, 24.6, 23.1, 21.1, 14.5, -5.1; CH_2 δ 93.1, 71.7, 69.3, 64.3, 36.1, 32.3; CH δ 138.1, 129.2, 128.3, 127.6 (×2), 124.1, 117.3, 113.7, 76.6, 72.1, 71.8, 68.3; CH_0 δ 169.0, 106.2, 159.0, 152.2, 137.8, 130.3, 102.5, 45.6, 18.3. IR (thin film) 2952, 2886, 2856, 1714, 1720, 1665, 1612, 1513, 1462, 1435, 1372, 1248, 1158, 1107, 943, 836, 777, 737, 698 cm^{-1}; HRMS (ESI) calcd for $C_{43}H_{64}NaO_{11}Si$ (M+Na) 807.4116, found 807.4129.



Preparation of (*E*)-methyl 2-((2*S*,3*S*,6*S*)-3-acetoxy-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-((*E*)-5-hydroxy-2-methylpent-3-en-2-yl)-2-methoxydihydro-2H-pyran-4(3H)-ylidene)acetate (2.195): To a stirring solution of the TBS ether **2.194** (495 mg, 0.63 mmol, 1.0 equiv) in a 5:4:1 THF/MeOH/ pyridine solution (13 mL, 0.05M) at 0 ºC in a

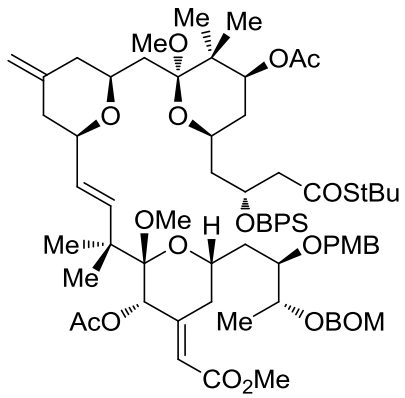
plastic bottle was added HF·py (13 mL of 20% in pyridine). The reaction mixture was stirred at 0 ºC for 10 min and then warmed to rt. Stirring was continued for 3 h and the reaction mixture was then quenched by pipetting it into a mixture of saturated aqueous $NaHCO_3$ solution and 50% EtOAc/hexanes (30 mL of each). The layers were separated and the aqueous layer was extracted with 50% EtOAc/hexanes (3 × 30 mL). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 2 × 8 cm silica gel column, eluting with 30% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (28-55) were combined and concentrated under reduced pressure to provide alcohol **2.195** (393 mg, 93%) as a colorless oil: R_f = 0.3 (50% EtOAc/hexanes); $[\alpha]_D^{20}$ = -5.9 (*c* = 1.1, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.19 (m, 7H), 6.85-6.82 (m, 2H), 5.98 (dt, *J* = 15.6, 1.6 Hz, 1H), 5.88 (bs, 1H), 5.49 (dt, *J* = 16.1, 5.8 Hz, 1H), 5.43 (s, 1H), 4.86 (d, *J* = 6.8 Hz, 1H), 4.84 (d, *J* = 6.8 Hz, 1H), 4.67 (s, 2H), 4.61 (d, *J* = 10.7 Hz, 1H), 4.41 (d, *J* = 11.2 Hz, 1H), 4.14 (dddd, *J* = 10.7, 6.3, 6.3, 3.7 Hz, 1H), 4.07-4.02 (m, 3H), 3.90 (ddd, *J* = 10.2, 4.3, 1.9 Hz, 1H), 3.78 (s, 3H), 3.69 (s, 3H), 3.46 (dd, *J* = 15.6, 2.4 Hz, 1H), 3.22 (s, 3H), 2.34 (ddd, *J* = 14.1, 10.9, 1.4 Hz, 1H), 2.05 (s, 3H), 1.92 (ddd, *J* = 14.1, 9.7, 1.9 Hz, 1H), 1.73 (ddd, *J* = 13.1, 10.2, 2.4 Hz, 1H), 1.45 (t, *J* = 6.2 Hz, 1H), 1.21 (d, *J* = 6.3 Hz, 3H), 1.12 (s, 3H), 1.10 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.1, 166.2, 158.9, 152.1, 138.9, 137.7, 130.2, 129.1, 128.2, 127.5 (×2), 124.6, 117.0, 113.5, 102.3, 93.0, 76.3, 71.9, 71.8, 71.4, 69.2, 68.2, 63.5, 54.9, 51.1, 50.9, 45.7, 35.9, 32.4, 23.9, 23.6, 21.0, 14.4; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 54.9, 51.1, 50.9, 23.9, 23.6, 21.0, 14.4; CH_2 δ 93.0, 71.4, 69.2, 63.5, 35.9, 32.4; CH δ 138.9, 129.1, 128.2, 127.5 (×2), 124.6,

117.0, 113.5, 76.3, 71.9, 71.7, 68.2; CH_0 δ 169.1, 166.2, 158.9, 152.1, 137.7, 130.2, 102.3, 45.7; IR (thin film) 2911, 2360, 1744. 1719, 1664, 1612, 1513, 1255, 1436, 1328, 1234, 1173, 846, 750, 698 cm^{-1}; HRMS (ESI) calcd for $C_{37}H_{50}NaO_{11}$ (M+Na) 693.3251, found 693.3248 .



Preparation of (*E*)-methyl 2-((2*S*,3*S*,6*S*)-3-acetoxy-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-methoxy-2-((*E*)-2-methyl-5-oxopent-3-en-2-yl)dihydro-2H-pyran-4(3H)-ylidene) acetate (2.146): To a stirring solution of the alcohol **2.195** (390 mg, 0.58 mmol, 1 equiv) in CH_2Cl_2 (12 mL) at rt, were added 4 Å molecular sieves (400 mg), TPAP (20 mg, 0.05 mmol, 0.1 equiv), and 4-methylmorpholine-N-oxide (204 mg, 1.74 mmol, 3.0 equiv). The mixture was stirred at rt for 1 h and then diluted with EtOAc (20 mL). The mixture was then filtered through a small plug of Florisil® and washed with copious amounts of EtOAc. The solvent was removed under reduced pressure and purification was accomplished by flash column chromatography, using a 2.5 × 7 cm silica gel column, eluting with 25% EtOAc/hexanes, collecting 6 mL fractions. The product containing fractions (18-35) were combined and concentrated under reduced pressure to provide aldehyde **2.146** (354 mg, 91%) as a colorless oil. R_f = 0.37 (40% EtOAc/hexanes); $[\alpha]_D^{20} =$-1.8 (*c* = 1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.50 (d, *J* = 7.3 Hz, 1H), 7.36-7.17 (m, 7H), 6.83-6.80 (m, 2H), 5.91 (dd, *J* = 16.1, 7.8 Hz, 1H), 5.86 (bs, 1H), 5.39

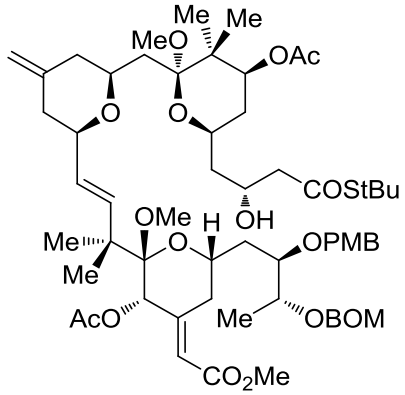
(s, 1H), 4.85 (d, *J* = 6.8 Hz, 1H), 4.82 (d, *J* = 6.8 Hz, 1H), 4.64 (s, 2H), 4.60 (d, *J* = 10.7 Hz, 1H), 4.38 (d, *J* = 11.2 Hz, 1H), 4.15 (dddd, *J* = 6.3, 6.3, 6.3, 4.1 Hz, 1H), 4.07 (dddd, *J* = 12.6, 9.5, 2.9, 2.9 Hz, 1H), 3.87 (ddd, *J* = 10.2, 4.3, 1.9 Hz, 1H), 3.77 (s, 3H), 3.68 (s, 3H), 3.51(dd, *J* = 16.1, 2.4 Hz, 1H), 3.23 (s, 3H), 2.34 (ddd, *J* = 14.1, 11.3, 1.4 Hz, 1H), 2.03-2.02 (m, 1H), 1.95 (ddd, *J* = 14.1, 9.3, 1.9 Hz, 1H), 1.89 (s, 3H), 1.75 (ddd, *J* = 13.1, 10.2, 2.9 Hz, 1H), 1.21 (d, *J* = 6.3 Hz, 3H), 1.13 (s, 3H), 1.11 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 194.1, 168.4, 166.4, 165.8, 159.0, 151.0, 137.6, 130.0, 129.0, 128.2, 127.4 (×2), 126.5, 117.6, 113.5, 102.1, 93.1, 76.1, 71.7, 71.2, 71.0, 69.2, 68.8, 54.9, 51.1, 50.9, 47.1, 35.8, 32.4, 23.6, 21.4, 20.9, 14.1; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 54.9, 51.1, 50.9, 23.6, 21.4, 20.9, 14.1; CH_2 δ 93.1, 71.2, 69.2, 35.8, 32.4; CH δ 194.1, 166.4, 129.1, 128.2, 127.4 (×2), 126.5, 117.5, 113.5, 76.1, 71.7, 71.0, 68.8; CH_0 δ 168.4, 165.8, 159.0, 151.0, 137.6, 130.0, 102.1, 47.1. IR (thin film) 2911, 2836, 1749, 1718, 1686, 1612, 1513, 1462, 1435, 1370, 1301, 1231, 1172, 1105, 1064, 904, 821, 752, 699 cm^{-1}; HRMS (ESI) calcd for $C_{37}H_{48}NaO_{11}$ (M+Na) 691.3094, found 691.3094.



Preparation of (*E*)-methyl 2-((2*S*,3*S*,6*S*)-3-acetoxy-2-((*E*)-4-((2*R*,6*S*)-6-(((2*S*,4*S*,6*S*)-4-acetoxy-6-((*R*)-2-((*tert*-butyldiphenyl silyl)oxy)-4-(*tert*-butylthio)-4-oxobutyl)-2-methoxy-3,3-dimethyltetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)-2-methylbut-3-en-2-yl)-

6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-methoxy dihydro-2H-pyran-4(3H)-ylidene)acetate (2.199): A flame dried 15 mL rb flask was charged with aldehyde **2.146** (101 mg, 0.151 mmol, 1.0 equiv) and hydroxyallylsilane **2.147** (130 mg, 0.166 mmol, 1.1 equiv) and subjected to high vacuum for 1 h. To this mixture was added Et_2O (2.1 mL) and the solution was cooled to -78 ºC with stirring under argon. To the stirring solution of aldehyde and silane was added a solution of TMSOTf in Et_2O (181 μL of 1.0 M, 0.181 mmol, 1.2 equiv). After 5 h at -78 ºC, the reaction mixture was quenched by the addition of diisopropylethylamine (200 μL) and stirred for 15 min. The mixture was warmed to 0 ºC and saturated aqueous $NaHCO_3$ solution (5 mL) was added. The mixture was warmed to rt, diluted with 5 mL Et_2O, and the phases were separated. The aqueous phase was extracted with Et_2O (3 × 10 mL). The organic phases were combined, washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 2 × 7 cm silica gel column, eluting with hexanes/EtOAc (9:1), collecting 4 mL fractions. The product containing fractions (42-60) were combined and concentrated under reduced pressure to provide the pyran **2.199** (123 mg, 60%) as a white foam. R_f = 0.36 (30% EtOAc/hexanes); $[\alpha]_D^{20} =$ +8.9 (*c* = 1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.65 (m, 4H), 7.44-7.29 (m, 11H), 7.21-7.19 (m, 2H), 6.84-6.82 (m, 2H), 5.99 (dd, *J* = 16.1, 0.9 Hz, 1H), 5.88 (s, 1H), 5.44 (s, 1H), 5.35 (dd, *J* = 16.1, 5.3 Hz, 1H), 4.95 (dd, *J* = 11.6, 4.8 Hz, 1H), 4.86 (d, *J* = 6.8 Hz, 1H), 4.84 (d, *J* = 6.8 Hz, 1H), 4.69 (s, 1H), 4.66 (s, 2H), 4.61(d, *J* = 11.2 Hz, 1H), 4.42 (d, *J* = 10.7 Hz, 1H), 4.27-4.23 (m, 1H), 4.13-4.05 (m, 2H), 3.88 (ddd, *J* = 9.7, 4.4, 1.9 Hz, 1H), 3.78 (s, 3H), 3.68 (s, 3H), 3.66-3.62 (m, 1H), 3.47 (dd, *J* = 13.6, 2.4 Hz, 1H), 3.39-3.37 (m, 1H), 3.23

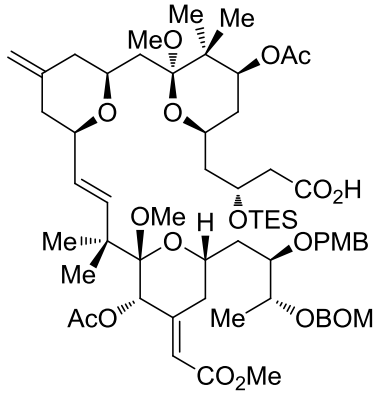
(s, 3H), 3.21-3.14 (m, 1H), 2.93 (s, 3H), 2.71-2.69 (m, 2H), 2.33 (ddd, *J* = 14.6, 12.1, 1.9 Hz, 1H), 2.27-2.10 (m, 2H), 2.05 (s, 3H), 1.99 (s, 3H), 1.93-1.79 (m, 4H), 1.74-1.62 (m, 4H), 1.44 (s, 9H), 1.32-1.26 (m, 2H), 1.21 (d, *J* = 6.3 Hz, 3H), 1.11 (s, 3H), 1.09 (s, 3H), 1.02 (s, 9H), 0.87 (s, 3H), 0.82 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 189.1, 170.6, 169.4, 166.6, 159.3, 152.6, 144.6, 138.0, 137.8, 136.1, 136.0, 134.4, 133.6, 130.6, 129.9, 129.8, 129.4, 128.6, 127.9 (×2), 127.8, 127.7, 126.5, 117.2, 114.0 (×2), 108.9, 104.0, 102.7, 93.5, 78.5, 76.9, 74.8, 73.7, 72.5, 72.1 (×2), 69.6, 69.4, 68.4, 66.0, 55.4, 53.1, 52.4, 51.3, 48.2, 46.1, 43.7, 42.5, 40.5, 39.1, 36.5, 33.0, 32.7, 30.0, 27.1, 24.3, 23.9, 21.5, 21.4, 20.7, 19.5, 16.7, 14.9; 125 MHz DEPT; CH_3 δ 55.4, 51.4, 51.3, 48.2, 29.9, 27.1, 24.3, 23.9, 21.5, 21.4, 20.7, 16.7, 14.9; CH_2 δ 108.8, 93.4, 72.1, 69.6, 53.1, 43.6, 42.5, 40.5, 39.0, 36.5, 33.0, 32.7; CH δ 137.7, 136.1, 135.9, 129.9, 129.8, 129.4, 128.6, 127.9, 127.7 (×2), 126.4, 117.2, 113.9 (×2), 78.5, 76.9, 74.8, 73.7, 72.5, 72.0, 69.3, 68.4, 66.0; CH_0 δ 189.1, 170.6, 169.4, 166.6, 159.3, 152.6, 144.6, 138.0, 134.4, 133.6, 130.6, 127.9, 104.0, 102.7, 46.1, 42.0, 19.5; IR (neat) 2951, 1743, 1721, 1681, 1598, 1513, 1460, 1429, 1365, 1246, 1163, 1109, 1040, 822, 755, 703 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{77}H_{106}NaO_{17}SSi$ (M+Na): 1385.6818, found:1385.6838.



Preparation of (*E*)-methyl 2-((2*S*,3*S*,6*S*)-3-acetoxy-2-((*E*)-4-((2*R*,6*S*)-6-(((2*S*,4*S*,6*R*)-4-acetoxy-6-((*R*)-4-(tert-butylthio)-2-

hydroxy-4-oxobutyl)-2-methoxy-3,3-dimethyltetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)-2-methylbut-3-en-2-yl)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl) oxy) butyl) -2-methoxydihydro-2H-pyran-4(3H)-ylidene)acetate (2.202): To a stirring solution of the BPS ether **2.199** (101 mg, 0.07 mmol, 1.0 equiv) in a 5:4:1 THF/MeOH/pyridine solution (3.7 mL, 0.02M) at 0 ºC in a 10 mL plastic centrifuge tube was added HF·py (1.6 mL of 20% in pyridine). The reaction mixture was stirred at 0 ºC for 1 h and then warmed to rt. Stirring was continued for 36 h and the reaction mixture was then quenched by pipetting it into a mixture of saturated aqueous $NaHCO_3$ solution and EtOAc (5 mL of each). The layers were separated and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 2 × 7 cm silica gel column, eluting with 25% EtOAc in hexanes, collecting 4 mL fractions. The product containing fractions (11-49) were combined and concentrated under reduced pressure to provide the alcohol **2.202** (75 mg, 90%) as a white foam. R_f = 0.3 (40% EtOAc/hexanes); $[\alpha]_D^{20} =$ +17.3 (*c* = 1.2, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.36-7.34 (m, 5H), 7.21-7.20 (m, 2H), 6.84-6.82 (m, 2H), 6.00 (dd, *J* = 16.1, 1.6 Hz, 1H), 4.78 (d, *J* = 7.5 Hz, 1H), 5.88 (s, 1H), 5.43 (s, 1H), 5.39 (dd, *J* = 16.1, 4.8 Hz, 1H), 5.19 (dd, *J* = 11.2, 4.8 Hz, 1H), 4.85 (d, *J* = 6.8 Hz, 1H), 4.70-4.68 (m, 2H), 4.67 (s, 2H), 4.61(d, *J* = 11.2 Hz, 1H), 4.42 (d, *J* = 11.2 Hz, 1H), 4.33-4.32 (m, 1H), 4.11 (ddd, *J* = 8.7, 7.3, 2.4 Hz, 1H), 4.08-4.05 (m, 1H), 3.96-3.92 (m, 1H), 3.89 (ddd, *J* = 10.5, 4.4, 2.2 Hz, 1H), 3.78 (s, 3H), 3.68 (s, 3H), 3.53-3.46 (m, 2H), 3.24 (s, 3H), 3.22 (s, 3H), 2.63-2.61 (m, 2H), 2.37-2.30 (m, 2H), 2.22-2.18 (m, 2H), 2.07 (s, 3H), 2.03 (s, 3H), 1.95-1.56 (m, 5H), 1.46 (s, 9H),

1.28-1.25 (m, 1H), 1.21 (d, *J* = 6.3 Hz, 3H), 1.11 (s, 3H), 1.10 (s, 3H), 1.01 (s, 3H), 0.91 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ; 200.1, 170.8, 169.4, 166.6, 159.3, 152.5, 144.7, 138.0, 137.7, 133.4, 130.6, 129.4, 128.6, 127.9, 127.8, 126.4, 117.2, 113.9, 108.8, 104.1, 102.7, 93.5, 78.6, 76.9, 75.0, 74.1, 72.5, 72.0, 69.6, 68.3, 65.3, 64.9, 55.4, 51.5, 51.4, 51.3, 48.5, 46.1, 42.4, 42.2, 41.9, 40.5, 39.2, 36.4, 32.9, 32.6, 29.9, 24.2, 24.1, 21.5, 21.4, 20.7, 17.0, 14.9.125 MHz DEPT; CH_3 δ; 55.4, 51.4, 51.3, 48.5, 29.9, 24.2, 24.1, 21.5, 21.4, 20.7, 17.0, 14.9 CH_2 δ; 108.8, 93.5, 72.0, 69.6, 51.5, 42.4, 41.9, 40.5, 39.2, 36.4, 32.9, 32.7, CH δ; 137.7, 129.4, 128.6, 127.9, 127.8, 126.4, 117.3, 113.9, 78.6, 76.9, 75.0, 74.1, 72.5, 72.1, 68.3, 65.3, 64.9 CH_0 δ; 200.1, 170.8, 169.4, 166.6, 159.3, 152.5, 144.7, 138.0, 133.4, 130.6, 104.1, 102.7, 46.1, 42.2; IR (neat) 2950, 2836, 1722, 1679, 1612, 1513, 1435, 1366, 1246, 1160, 1105, 1041, 982, 900, 849, 755, 699 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{61}H_{88}NaO_{17}S$ (M+Na): 1147.5635, found:1147.5652.

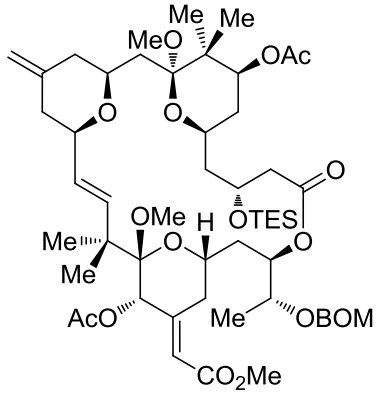


Preparation of (*R*)-4-((2*S*,4*S*,6*S*)-4-acetoxy-6-(((2*S*,6*R*)-6-((*E*)-3-((2*S*,3*S*,6*S*,*E*)-3-acetoxy-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl) oxy)butyl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2H-pyran-2-yl)-3-methylbut-1-en-1-yl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)-6-methoxy-5,5-dimethyltetrahydro-2H-pyran-2-yl)-3-((triethylsilyl) oxy) butanoic

acid (2.203): To a stirring solution of thiolester **2.202** (32 mg, 0.0284 mmol, 1.0 equiv) in THF (2.4 mL) in a 5 mL vial at 0 ºC were added water (0.6 mL), LiOH powder (14 mg, 0.568 mmol, 20 equiv) and H_2O_2 (0.64 mL of 30%, 0.568 mmol, 20 equiv). The resulting solution was stirred at 0 °C for 1 h, then poured into a mixture of aqueous pH 6 phosphate buffer solution and EtOAc (5 mL of each). The layers were separated and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the hydroxy acid as a sticky oil, which was taken into the next step without further purification.

To a stirring solution of this hydroxy acid in CH_2Cl_2 (1.4 mL) in a 5 mL vial at -15 ºC was added DMAP (35 mg, 0.284 mmol, 10 equiv), followed by TESCl (23 µL, 0.142 mmol, 5 equiv) via syringe. The solution was stirred at -15 ºC for 1 h, then poured into a mixture of aqueous pH 4 (acetic acid/sodium acetate) buffer and EtOAc (5 mL of each). The layers were separated and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 1 × 5 cm silica gel column, eluting with 30% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (3-12) were combined and concentrated under reduced pressure to provide the carboxylic acid **2.203** (24.8 mg, 77% over 2 steps) as a white foam: R_f = 0.66 (5:4:1 hexanes: EtOAc:MeOH); $[\alpha]_D^{20} = +13$ (*c* = 1.1, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.36-7.22 (m, 5H), 7.21-7.19 (m, 2H), 6.84-6.82 (m, 2H), 6.00 (d, *J* = 14.6 Hz, 1H), 5.88 (s, 1H), 5.43 (s, 1H), 5.37 (dd, *J* = 16.1, 5.3 Hz, 1H), 5.15 (dd, *J* = 11.7, 4.3 Hz, 1H), 4.85 (s, 2H), 4.71-4.68 (m, 2H), 4.66 (s, 2H), 4.60 (d, *J* = 10.7 Hz, 1H), 4.42 (d, *J* = 10.7 Hz, 1H), 4.25-4.22 (m, 1H), 4.13-4.05 (m,

2H), 3.89 (ddd, *J* = 10.2, 4.8, 2.6 Hz, 1H), 3.77 (s, 3H), 3.68 (s, 3H), 3.52-3.46 (m, 1H), 3.23 (s, 3H), 3.18 (s, 3H), 2.68 (dd, *J* = 15.1, 4.3 Hz, 1H), 2.52 (dd, *J* = 15.1, 6.8 Hz, 1H), 2.35-2.27 (m, 2H), 2.21-2.11 (m, 2H), 2.07 (s, 3H), 2.03 (s, 3H), 1.96-1.61 (m, 9H), 1.44-1.37 (m, 1H), 1.21 (d, *J* = 6.3 Hz, 3H), 1.11 (s, 3H), 1.09 (s, 3H), 1.00 (s, 3H), 0.94 (t, *J* = 7.8 Hz, 9H), 0.91 (s, 3H), 0.60 (q, *J* = 7.8 Hz, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ; 170.9, 169.4, 166.6, 159.3, 152.5, 144.6, 137.9, 137.8, 129.4, 128.6, 127.9, 127.8, 126.5, 117.2, 113.9, 108.8, 104.2, 102.7, 93.4, 78.7, 76.9, 74.8, 74.0, 72.5, 72.0, 69.6, 68.4, 68.0, 67.8, 65.8, 55.4, 51.4, 51.3, 48.7, 48.4, 46.1, 44.2, 43.3, 42.4, 42.1, 40.6, 39.2, 36.4, 33.3, 33.0, 24.2, 24.1, 23.9, 21.5, 21.4, 20.8, 17.2, 16.9, 14.8, 7.0, 5.2; 125 MHz DEPT; CH_3 δ; 55.4, 51.4, 51.3, 48.6, 48.4, 24.2, 23.9, 21.5, 21.4, 20.8, 16.9, 14.8, 7.0; CH_2 δ; 108.7, 93.3, 72.0, 69.6, 44.2, 43.3, 42.4, 40.6, 39.1, 36.4, 33.3, 33.0, 5.2; CH δ; 137.8, 129.4, 128.5, 127.9, 127.8, 126.5, 117.2, 113.9, 78.7, 76.8, 74.8, 74.0, 72.5, 72.0, 68.4, 67.8, 65.8; CH_0 δ 170.9, 169.4, 166.6, 159.3, 152.5, 144.6, 137.9, 130.9, 130.6, 104.2, 68.0, 46.1, 42.1, 24.1, 17.2; IR (neat) 2953, 2877, 1721, 1612, 1513, 1459, 1379, 1245, 1172, 1081, 1041, 848, 740 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{62}H_{91}NaO_{17}Si$ (M+Na): 1189.6107, found:1189.6110.



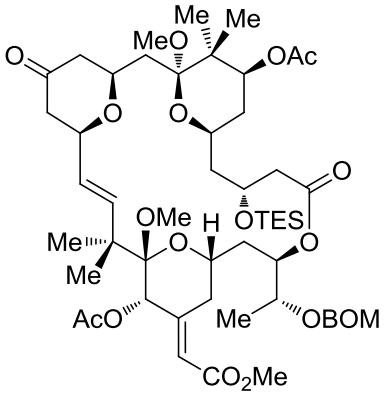
Preparation of (3*S*,7*R*,8*E*,11*S*,12*S* ,13*E*,15*S*,17*R* ,21*R*,23*S*,25*S*)-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-1,11-dimethoxy-13-(2-methoxy-

2-oxoethylidene)-10,10,26,26-tetramethyl-5-methylene-19-oxo-21-((triethylsilyl) oxy)-18,27,28,29-tetraoxatetracyclo[21.3.1.13,7.111,15]nonacos-8-ene-12,25-diyl diacetate (2.204): To a stirring solution of the PMB ether **2.203** (45.2 mg, 0.0397 mmol, 1 equiv) in CH_2Cl_2 (3.2 mL) at 0 ºC was added pH 8 phosphate buffer (800 µL). DDQ (45 mg, 0.198 mmol, 5 equiv) was added in one portion and the reaction was stirred vigorously for 1.5 h. The reaction mixture was then poured into a mixture of CH_2Cl_2 and pH 4 acetate buffer (5 mL each), the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude product was quickly passed through a column of silica gel (1 × 7 cm) eluting with 30% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions 8-19 were combined and concentrated under reduced pressure to give the seco acid, partially mixed with DDQ byproducts, which was taken to the next step without further purification.

To a stirring solution of the seco acid in THF (1.3 mL) at 0 °C were added triethylamine (31 µL, 0.238 mmol, 6.0 equiv) and 2,4,6-trichlorobenzoyl chloride (18 µL, 0.119 mmol, 3.0 equiv) via syringe. After 10 min, the reaction mixture was warmed to rt and stirring was continued for an additional 4 h. The reaction mixture was then diluted with 3:1 toluene/THF (20 mL) and taken up into a 25 mL gas-tight syringe. This solution was added by syringe pump to a stirring solution of DMAP (97 mg, 0.795 mmol, 20.0 equiv) in toluene (39 mL) at 40 °C over 12 h. The residual contents of the syringe were rinsed into the flask with toluene (0.5 mL) and stirring was continued for an additional 2 h. The reaction mixture was cooled to rt, diluted with 30% EtOAc/hexanes (30 mL), and washed with saturated aqueous $NaHCO_3$ solution (10 mL) and with brine (10 mL). The

organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography with a 1 × 8 cm silica gel column, eluting with 20% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (5-12) were combined and concentrated under reduced pressure to provide macrolactone **2.204** as a white foam (28 mg, 71% over 2 steps). R_f = 0.46 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +54.4 (*c* = 1.0, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.31 (m, 5H), 6.27 (d, *J* = 15.6 Hz, 1H), 5.95 (d, *J* = 1.9 Hz, 1H), 5.65 (dd, *J* = 10.2, 4.8 Hz, 1H), 5.33 (dd, *J* = 16.1, 8.3 Hz, 1H), 5.20 (dd, *J* = 11.7, 4.8 Hz, 1H), 5.18 (s, 1H), 4.85 (d, *J* = 6.8 Hz, 1H), 4.83 (d, *J* = 6.8 Hz, 1H), 4.72-4.71 (m, 2H), 4.67 (s, 2H), 4.45 (ddd, *J* = 12.8, 9.4, 4.0 Hz, 1H), 3.99 (ddd, *J* = 10.5, 7.8, 2.1 Hz, 1H), 3.85 (dd, *J* = 5.8, 4.8 Hz, 2H), 3.78-3.73 (m, 2H), 3.69 (s, 3H), 3.11 (s, 3H), 3.10 (s, 3H), 2.63 (dd, *J* = 17.5, 3.4 Hz, 1H), 2.19 (dd, *J* = 17.5, 9.2 Hz, 2H), 2.18-2.08 (m, 4H), 2.06 (s, 3H), 2.02 (s, 3H), 1.87-1.82 (m, 1H), 1.69-1.61 (m, 4H), 1.28-1.18 (m, 7H), 1.10 (s, 3H), 1.08 (s, 3H), 0.94 (t, *J* = 6.8 Hz, 9H), 0.90-0.87 (m, 1H), 0.85 (s, 3H), 0.58 (dq, *J* = 7.3, 1.9 Hz, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ; 170.9. 170.7, 169.3, 166.9, 151.3, 145.1, 139.2, 137.8, 128.7, 128.0 (×2), 127.2, 119.5, 108.5, 103.1, 93.4, 79.8, 74.2, 74.0, 73.7, 73.4, 70.8, 69.9, 66.8, 65.9, 64.6, 53.3, 51.4, 48.3, 45.4, 44.9, 43.5, 41.7, 41.4, 40.9, 40.2, 36.6, 33.9, 30.9, 26.6, 21.6, 21.5, 20.5, 20.4, 17.6, 16.1, 7.2, 5.8, 1.2; 125 MHz DEPT; CH_3 δ; 53.3, 51.4, 48.3, 26.6, 21.6, 21.5, 20.4, 17.6, 16.1, 7.2, 1.2; CH_2 δ; 108.5, 93.4, 69.9, 45.4, 43.5, 41.4, 40.9, 40.1, 36.6, 33.9, 30.9, 5.8; CH δ; 139.3, 128.7, 128.0 (×2), 127.2, 119.6, 79.8, 74.2, 74.0, 73.7, 73.4, 70.8, 66.8, 65.9, 64.6; CH_0 δ 170.9, 170.7, 169.3, 166.9, 151.3, 145.1, 137.8, 103.1, 44.9, 41.7, 20.5; IR (neat) 2951, 2878, 1735, 1653, 1595,

1456, 1434, 1368, 1238, 1163, 1100, 1027, 890, 745 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{54}H_{81}NaO_{15}Si$ (M+Na): 1051.5426, found:1051.5442.

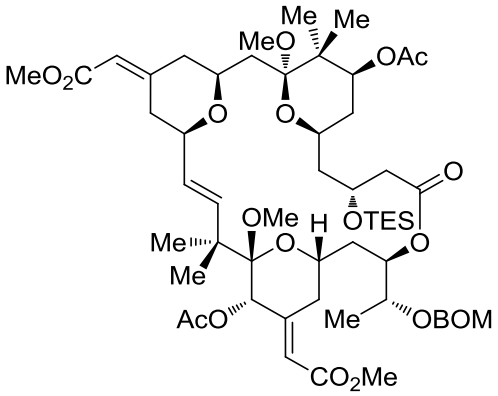


Preparation of (3*S*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*S*,25*S*)-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-1,11-dimethoxy-13-(2-methoxy-2-oxoethylidene)-10,10,26,26-tetramethyl-5,19-dioxo-21-((triethylsilyl)oxy)-18,27,28,29-tetraoxatetracyclo [21.3.1.13,7.111,15] nonacos-8-ene-12,25-diyl diacetate (2.205). To a stirring solution of olefin **2.204** (21.1 mg, 0.021 mmol**,** 1.0 equiv) in *t*-butanol (0.5 mL) and water (0.5 mL) at rt was added AD mix-α (29 mg, 1.4 g/mmol olefin). The reaction was allowed to proceed for 24 h, after which time TLC analysis indicated completion of reaction. The reaction mixture was quenched by the addition of saturated aqueous sodium bisulfite solution (5 mL) and the resulting mixture was stirred for 30 min, then diluted with EtOAc (5 mL) and the layers were separated. The aqueous layer was extracted with EtOAc (2 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the diol as a colorless paste which was taken directly onto the next reaction without further purification.

To a stirring solution of the crude diol in THF (0.75 mL) and 0.1 M pH 7 phosphate buffer (0.25 mL) at rt was added $NaIO_4$ (23 mg, 0.105 mmol, 5 equiv). The mixture was

stirred vigorously at rt for 3 h, then diluted with EtOAc (5 mL) and quenched by the addition of saturated aqueous $NaHCO_3$ solution (5 mL) and the layers were separated. The aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography using a 1 × 5 cm silica gel column, eluting with 30% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (3-7) were combined and concentrated under reduced pressure to provide ketone **2.205** as a white foam (17.7 mg, 81% over 2 steps). R_f = 0.5 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +88.2 (*c* = 0.88, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.26 (m, 5H), 6.35 (d, *J* = 15.6 Hz, 1H), 5.95 (d, *J* = 1.4 Hz, 1H), 5.62 (dd, *J* = 10.7, 4.3 Hz, 1H), 5.35 (dd, *J* = 16.1, 7.8 Hz, 1H), 5.20 (dd, *J* = 10.2, 4.8 Hz, 1H), 5.20 (s, 1H), 4.85 (d, *J* = 6.8 Hz, 1H), 4.82 (d, *J* = 6.8 Hz, 1H), 4.66 (s, 2H), 4.41-4.33 (m, 2H), 4.10-4.06 (m, 1H), 3.84-3.81 (m, 2H), 3.75-3.71 (m, 1H), 3.70 (s, 3H), 3.66-3.60 (m, 2H), 3.10 (s, 3H), 3.08 (s, 3H), 2.66 (dd, *J* = 17.5, 2.9 Hz, 1H), 2.40-2.16 (m, 7H), 2.06 (s, 3H), 2.03 (s, 3H), 1.85-1.80 (m, 1H), 1.66-1.63 (m, 3H), 1.23 (s, 3H), 1.22 (s, 3H), 1.10 (s, 3H), 1.08 (s, 3H), 0.98 (s, 3H), 0.93 (t, *J* = 8.3 Hz, 9H), 0.57 (q, *J* = 8.3 Hz, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ; 207.7, 170.8, 170.7, 169.3, 166.8, 151.1, 140.5, 128.7, 128.0, 125.5, 119.6, 103.0, 102.9, 93.5, 78.4, 77.4, 74.4, 73.5, 72.2, 71.0, 69.9, 66.8, 65.8, 64.7, 53.2, 51.4, 48.6, 48.0 (×2), 45.1, 44.9, 43.1, 41.7, 39.9, 36.9, 33.8, 30.8, 29.9, 26.7, 21.6, 21.5, 20.5, 20.3, 17.5, 16.4, 7.2, 5.8; 125 MHz DEPT; CH_3 δ; 53.2, 51.4, 48.0, 26.7, 21.6, 21.5, 20.5, 20.3, 17.5, 16.4, 7.2; CH_2 δ; 93.5, 69.9, 48.6, 48.1, 45.1, 43.1, 39.9, 36.9, 33.8, 30.8, 5.8; CH δ; 140.5, 128.7, 128.0, 125.5, 119.6, 78.4, 77.4, 74.4, 74.0, 73.5, 72.2, 71.0, 66.8, 65.8, 64.7; CH_0 δ 207.7, 170.8, 170.7, 169.3, 166.8, 151.1,

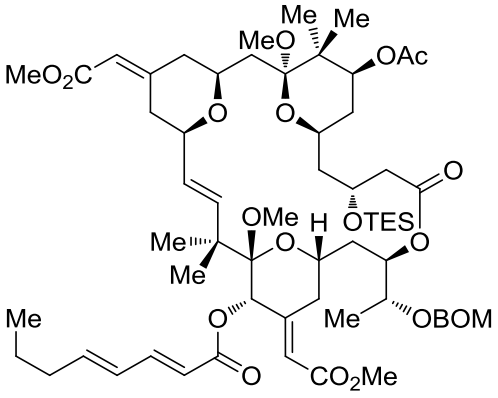
103.0, 44.9, 41.7, 36.9, 29.9; IR (neat) 2953, 2879, 1729, 1667, 1596, 1457, 1434, 1368, 1239, 1163, 1097, 1027, 903, 751 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{54}H_{81}NaO_{15}Si$ (M+Na): 1053.5219, found:1053.5208



Preparation of (2*Z*,2'*E*)-dimethyl 2,2'-((3*S*,7*R*,11*S*,12*S*,15*S*,17*R*,21*R*,23*S*,25*S*,*E*)-12,25-diacetoxy-17-((*R*)-1-((benzyloxy) methoxy)ethyl)-1,11-dimethoxy-10,10,26,26-tetramethyl-19-oxo-21-((triethylsilyl) oxy)-18,27,28,29- tetraoxatetracyclo [21.3.1.13,7.111,15] nonacos-8-ene-5, 13-diylidene) diacetate (2.207). To a stirring solution of the *R*-BINOL phosphonate **2.206** (70 mg, 0.174 mmol, 15 equiv) in THF (1 mL) in a 5 mL vial at -78 °C was added a solution of NaHMDS in THF (117 µL of 1.0 M, 0.116 mmol, 10 equiv) down the wall of the vial via syringe. The resulting solution was stirred at -78 °C for 30 min. The ketone **2.205** (12 mg, 0.0116 mmol, 1 equiv) was dissolved in THF (100 µL) and added dropwise along the side of the vial via syringe and then rinsed in with THF (0.1 mL). The solution was stirred at -78 °C for 1 h then warmed to 0 °C and stirring was continued for 48 h. The reaction was quenched by the dropwise addition of saturated aqueous $NaHCO_3$ solution (5 mL) and was then diluted with EtOAc (5 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification

was accomplished using flash column chromatography with a 1 × 5 cm silica gel column, eluting with 30% EtOAc/hexanes, collecting 4 mL fractions. The product containing fractions (3-6) were combined and concentrated under reduced pressure to provide the desired unsaturated ester as a 4:1 *Z*:*E* mixture of diastereomers. The *E* and *Z* diastereomers were separated using preparative thin layer chromatography eluting with 10% EtOAc/benzene to afford 8.5 mg of the desired *Z* isomer **2.207** and 2.1 mg of the *E* isomer (combined yield 85%), both as colorless oils. Analytical data for the desired *Z* isomer: R_f = 0.39 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +92.3 (*c* = 0.53, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.29 (m, 5H), 6.30 (d, *J* = 15.6 Hz, 1H), 5.95 (d, *J* = 1.4 Hz, 1H), 5.69 (s, 1H), 5.62 (dd, *J* = 10.2, 4.3 Hz, 1H), 5.35 (dd, *J* = 15.6, 7.8 Hz, 1H), 5.19 (dd, *J* = 11.7, 4.8 Hz, 1H), 5.17 (s, 1H), 4.86 (d, *J* = 7.3 Hz, 1H), 4.83 (d, *J* = 6.8 Hz, 1H), 4.68 (d, *J* = 11.7 Hz, 1H), 4.64 (d, *J* = 11.7 Hz, 1H), 4.40 (dddd, *J* = 9.7, 9.7, 2.7, 2.7 Hz, 1H), 4.03 (ddd, *J* = 10.7, 7.8, 2.3 Hz, 1H), 3.88-3.72 (m, 5H), 3.69 (s, 3H), 3.69 (s, 3H), 3.67-3.59 (m, 2H), 3.10 (s, 3H), 3.09 (s, 3H), 2.64 (dd, *J* = 17.5, 3.4 Hz, 1H), 2.37 (dd, *J* = 17.5, 9.7 Hz, 1H), 2.18-2.10 (m, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01-1.98 (m, 2H), 1.92 (t, *J* = 12.6 Hz, 1H), 1.83 (t, *J* = 11.7 Hz, 1H), 1.67-1.62 (m, 4H), 1.24 (s, 3H), 1.23 (d, *J* = 6.3 Hz, 3H), 1.15-1.14 (m, 1H), 1.11 (s, 3H), 1.08 (s, 3H), 0.95 (s, 3H), 0.93 (t, *J* = 7.8 Hz, 9H), 0.85 (s, 3H), 0.57 (dq, *J* = 16.1, 2.4 Hz, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ; 170.9, 170.7, 169.3, 167.1, 166.9, 158.4, 151.3, 139.9, 137.8, 128.6, 128.0, 127.9, 126.7, 119.5, 114.4, 103.1, 103.0, 93.6, 78.9, 74.5, 74.0, 73.7, 72.9, 71.0, 69.9, 66.8, 65.9, 64.7, 53.3, 51.4, 51.1, 48.2, 45.2, 45.0, 43.3, 42.6, 41.7, 40.1, 37.0, 36.5, 33.9, 30.8, 26.5, 21.6, 21.4, 20.5 (×2), 17.5, 16.4, 7.1, 5.8; 125 MHz DEPT; CH_3 δ; 53.3, 51.4, 51.1, 48.2, 26.5, 21.6, 21.4, 20.4 (×2), 17.5, 16.4, 7.1; CH_2 δ; 93.6, 69.9, 45.2, 43.3, 42.6, 40.1, 37.0,

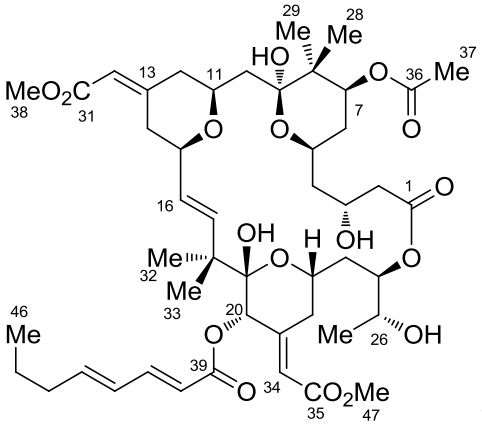
36.5, 33.9, 30.8, 5.8; CH δ; 139.9, 128.6, 128.0, 127.9, 126.7, 119.5, 114.4, 78.9, 74.5, 74.0, 73.7, 72.8, 71.0, 66.8, 65.9, 64.7; CH_0 δ 170.9, 170.7, 169.3, 167.1, 166.9, 158.4, 151.3, 137.8, 103.1, 103.0, 47.7, 45.0; IR (neat) 2952, 1740, 1612, 1513, 1456, 1382, 1249, 1173, 1107, 1039, 835, 739, 699 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{57}H_{86}NaO_{18}Si$ (M+Na): 1109.5481, found:1109.5493.



Preparation of (2*Z*,2'*E*)-dimethyl 2,2'-((3*S*,7*R*,11*S*,12*S*,15*S*,17*R*,21*R*,23*S*,25*S*,*E*)-25-acetoxy-17-((*R*)-1-((benzyloxy) methoxy)ethyl)-1,11-dimethoxy-10,10,26,26-tetramethyl-12-((2*E*,4*E*)-octa-2,4-dienoyloxy)-19-oxo-21-((triethylsilyl)oxy)-18,27,28,29-tetraoxatetracyclo [21.3.1.13,7.111,15]nonacos-8-ene-5,13-diylidene)diacetate (2.208) To a stirring solution of diacetate **2.207** (4.7 mg, 0.00432 mmol, 1.0 equiv) in MeOH (400 µL) in a 4 mL vial at rt was added K_2CO_3 (3 mg, 0.0216 mmol, 5 equiv) and the mixture was stirred for 45 min. The reaction mixture was quenched by the addition of saturated aqueous NH_4Cl solution (5 mL) and was then diluted with CH_2Cl_2 (5 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phases were washed with brine (5 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. This intermediate unstable alcohol was taken into the following reaction without further purification.

To a stirring solution of this alcohol in CH_2Cl_2 (400 µL, 0.001 M) in a 5 mL reaction vial at rt were added pyridine (17 µL, 0.21 mmol, 50 equiv), DMAP (5.0 mg, 0.043 mmol, 10 equiv), and octadienoic anhydride (35 mg, 0.129 mmol, 30 equiv). The reaction mixture stirred at rt for 12 h and was then quenched by the addition of saturated aqueous $NaHCO_3$ solution (5.0 mL). The mixture was stirred vigorously for 30 min and was then diluted with 40% EtOAc/hexanes (5 mL). The phases were separated and the aqueous phase was extracted with 40% EtOAc/hexanes (2 × 5 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography on a 1 × 3 cm silica gel column eluting with 10% EtOAc/hexanes (12 mL) followed by 30% EtOAc/hexanes, collecting 4 mL fractions. Fractions 7-9 provided the desired product **2.208** as a pale yellow oil (3.6 mg, 71% over 2 steps) R_f = 0.46 (30% EtOAc/hexanes); $[\alpha]_D^{20}$ = +53.7 (*c* = 0.18, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.28 (m, 5H), 6.31 (d, *J* = 16.1 Hz, 1H), 6.18-6.17 (m, 2H), 5.98 (d, *J* = 1.9 Hz, 1H), 5.78 (d, *J* = 15.1 Hz, 1H), 5.69 (s, 1H), 5.64 (ddd, *J* = 11.7, 4.8, 1.7 Hz, 1H), 5.34 (dd, *J* = 16.1, 7.8 Hz, 1H), 5.26 (s, 1H),), 5.20 (dd, *J* = 11.7, 4.8 Hz, 1H), 4.87 (d, *J* = 7.3 Hz, 1H), 4.84 (d, *J* = 6.8 Hz, 1H), 4.68 (d, *J* = 11.7 Hz, 1H), 4.65 (d, *J* = 11.7 Hz, 1H), 4.43-4.39 (m, 1H), 4.05-4.02 (m, 1H), 3.88-3.71 (m, 6H), 3.68 (s, 3H), 3.63-3.61 (m, 2H), 3.10 (s, 3H), 3.10 (s, 3H), 2.64 (dd, *J* = 17.0, 3.4 Hz, 1H), 2.38 (dd, *J* = 17.5, 9.7 Hz, 1H), 2.32-2.30 (m, 1H), 2.16-2.09 (m, 5H), 2.02 (s, 3H), 1.95-1.81 (m, 3H), 1.66-1.62 (m, 3H), 1.50-1.43 (m, 3H), 1.25 (s, 3H), 1.23 (s, 3H), 1.11 (s, 3H), 1.09 (s, 3H), 0.95-0.91 (m, 15H), 0.85 (s, 3H), 0.85 (s, 3H), 0.57 (dq, *J* = 18.0, 2.4 Hz, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ; 170.9, 170.7, 167.1, 167.0, 158.5, 151.6, 146.8, 145.9, 140.0, 137.8, 128.6, 128.5, 128.0, 127.9, 126.6, 119.4, 118.5, 114.3,

103.3, 103.0, 93.6, 78.9, 74.5, 74.1, 73.4, 72.9, 71.0, 69.9, 66.8, 66.0, 64.7, 53.3, 51.3, 51.0, 48.2, 45.2, 45.1, 43.3, 42.6, 41.7, 40.1, 37.1, 36.5, 35.2, 34.5, 33.9, 31.0, 26.6, 22.0, 21.4, 20.5, 20.4, 17.5, 16.4, 13.9, 7.1, 5.8; 125 MHz DEPT; CH_3 δ; 53.3, 51.3, 51.1, 48.2, 26.6, 21.4, 20.5, 17.5, 16.4, 13.9, 7.1; CH_2 δ; 93.6, 69.9, 45.2, 43.3, 42.6, 40.1, 37.1, 36.5, 35.2, 33.9, 30.9, 22.0, 5.8; CH δ; 146.8, 145.9, 140.0, 128.6, 128.5, 128.0, 127.9, 126.6, 119.4, 118.5, 114.3, 78.9, 74.5, 74.0, 73.4, 72.8, 71.0, 66.8, 65.9, 64.7; CH_0 δ; 170.9, 170.7, 167.1, 167.0, 158.5, 151.6, 137.8, 103.3, 103.0, 45.1, 41.7, 34.5; IR (neat) 2958, 2857, 1737, 1680, 1613, 1514, 1471, 1427, 1365, 1247, 1173, 1109, 834, 740, 704, 611 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{63}H_{94}NaO_{18}Si$ (M+Na): 1189.6107, found:1189.6110.



Preparation of Bryostatin 1: To a 4 mL reaction vial containing the protected bryostatin 1 **29** (3.6 mg, 0.003 mmol, 1 equiv) was added a solution of $LiBF_4$ in 25:1 CH_3CN/water (550 µL of 0.25 M, 0.003 mmol, 45.0 equiv). The reaction vial was sealed and the mixture was stirred at 80 ºC for 14 h. After cooling to rt, the reaction mixture was diluted with EtOAc (5 mL) and was quenched by the addition of saturated aqueous $NaHCO_3$ solution (5 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 × 5 mL). The combined organic

phases were dried over Na_2SO_4, filtered and concentrated. Purification was accomplished using flash column chromatography with a 0.5 × 6 cm silica gel column, collecting 6 × 50 mm test tube fractions, eluting with 20% EtOAc/hexanes for fractions from 1-10 followed by 50% EtOAc/hexanes. The product containing fractions (21-40) were combined and concentrated under reduced pressure to provide bryostatin 1 (2.0 mg, 72%) as a white powder. R_f = 0.37 (60% EtOAc/hexanes). $[\alpha]_D^{20} =$ +11 (*c* = 0.20, $CHCl_3$), natural $[\alpha]_D^{20} =$ +9 (*c* = 0.21, $CHCl_3$), IR (neat) 2934, 1716, 1643, 1435, 1366, 1246, 1160, 1099, 1003, 860, 812, 754, 733 cm^{-1}; HRMS (ESI/APCI) calcd for $C_{47}H_{68}NaO_{17}$ (M+Na): 927.4354, found: 927.4355; 500 MHz ^1H, 125 Mz ^{13}C, and DEPT in $CDCl_3$ are shown in Table **2.1**, **2.2** and **2.3**, respectively.

Table 2.1. Comparison of Proton NMR Chemical Shifts for Bryostatin 1

Proton No	Natural (a)	Natural (b)	Synthetic (b)
2a	2.42 (dd, J = 12.2, 2.1 Hz 1H)	2.45 (dd, J = 12.2, 2.0 Hz 1H)	2.45 (dd, J = 12.2, 2.0 Hz 1H)
2b	2.53 (dd, J = 12.2, 12.0 Hz 1H)	2.51 (dd, J = 12.2, 10.2 Hz 1H)	2.51 (dd, J = 12.2, 10.2 Hz 1H)
3	4.12 (m, 1H)	4.15 (m, 1H)	4.16 (m, 1H)
3-OH	4.24 (d, J = 12.0 Hz, 1H)	4.24 (m, 1H)	4.25 (m, 1H)
4a	1.55 (ddd, J = 14.8, 3.6, 3.1 Hz, 1H)	1.58 (ddd, J = 14.6, 3.3, 3.0 Hz, 1H)	1.57 (ddd, J = 15.1, 3.8, 3.1 Hz, 1H)
4b	2.02 (m, 1H)	2.05 (m, 1H)	2.06 (m, 1H)
5	4.21 (tt, J = 11.6, 2.3 Hz, 1H)	4.22 (tt, J = 11.7, 2.6 Hz, 1H)	4.23 (tt, J = 11.7, 1.5 Hz, 1H)
6ax	1.48 (q, J = 11.7 Hz, 1H)	1.47 (q, J = 10.7 Hz, 1H)	1.48 (q, J = 10.7 Hz, 1H)
6eq	1.72 (ddd, J = 12.3, 4.6, 2.6 Hz, 1H)	1.75 (ddd, J = 12.7, 4.9, 2.8 Hz, 1H)	1.75 (ddd, J = 12.6, 4.9, 2.9 Hz, 1H)
7	5.19 (m, 1H)	5.19 (m, 1H)	5.19 (m, 1H)
9-OH	3.25 (br, 1H)	2.97 (br, 1H)	2.92 (br, 1H)
10a	1.66 (d, J = 14.9 Hz, 1H)	1.66 (d, J = 15.1 Hz, 1H)	1.67 (d, J = 15.1 Hz, 1H)
10b	2.06 (m, 1H)	2.06 (m, 1H)	2.06 (m, 1H)
11	3.96 (ddd, J = 11.2, 7.5, 2.2 Hz, 1H)	3.86 (ddd, J = 10.2, 7.1, 2.3 Hz, 1H)	3.86 (ddd, J = 9.7, 7.1, 1.8 Hz, 1H)
12ax	2.22 (t, J = 12 Hz, 1H)	2.21 (t, J = 12.2 Hz, 1H)	2.21 (t, J = 12.2 Hz, 1H)
12eq	2.10 (m, 1H)	2.10 (m, 1H)	2.10 (m, 1H)
14ax	1.87 (br, 1H)	1.88 (br, 1H)	1.88 (br, 1H)
14eq	3.66 (m, 1H)	3.67 (m, 1H)	3.67 (m, 1H)
15	4.09 (ddd, J = 11.0, 8.5, 2.5 Hz, 1H)	4.09 (ddd, J = 10.7, 8.1, 1.9 Hz, 1H)	4.08 (ddd, J = 10.2, 7.6, 1.3 Hz, 1H)
16	5.32 (dd, J = 15.7, 8.5 Hz, 1H)	5.32 (dd, J = 15.6, 8.3 Hz, 1H)	5.32 (dd, J = 15.6, 8.3 Hz, 1H)
17	5.78 (d, J = 15.6 Hz, 1H)	5.78 (d, J = 15.6 Hz, 1H)	5.78 (d, J = 16.1 Hz, 1H)
19-OH	5.15 (s, 1H)	5.16 (s, 1H)	5.16 (s, 1H)
20	5.18 (s, 1H)	5.19 (s, 1H)	5.19 (s, 1H)
22ax	2.06 (m, 1H)	2.06 (m, 1H)	2.06 (m, 1H)
22eq	3.69 (m, 1H)	3.68 (m, 1H)	3.68 (m, 1H)

Table 2.1. Continued

Proton No	Natural (a)	Natural (b)	Synthetic (b)
23	4.02 (tt, J = 11.3, 2.4 Hz, 1H)	4.02 (tt, J = 11.2, 2.3 Hz, 1H)	4.02 (tt, J = 11.2, 2.1 Hz, 1H)
24a	1.83 (ddd, J = 13.8, 11.6, 2.9 Hz, 1H)	1.83 (ddd, J = 14.4, 11.5, 2.7 Hz, 1H)	1.83 (ddd, J = 14.4, 11.3, 3.2 Hz, 1H)
24b	1.99 (m, 1H)	1.98 (m, 1H)	1.97 (m, 1H)
25	5.17 (m, 1H)	5.18 (m, 1H)	5.18 (m, 1H)
26	3.78 (m, 1H)	3.79 (m, 1H)	3.78 (m, 1H)
27	1.24 (d, J = 6.4 Hz)	1.24 (d, J = 6.3 Hz)	1.24 (d, J = 6.8 Hz)
27-OH	3.75 (n.d.)	(n.d.)	(n.d.)
28	1.00 (s, 3H)	1.01 (s, 3H)	1.01 (s, 3H)
29	0.96 (s, 3H)	0.95 (s, 3H)	0.95 (s, 3H)
30	5.68 (t, J = 1.7 Hz, 1H)	5.68 (s, 1H)	5.68 (s, 1H)
32	1.15 (s, 3H)	1.15 (s, 3H)	1.16 (s, 3H)
33	1.01 (s, 3H)	1.01 (s, 3H)	1.01 (s, 3H)
34	6.00 (d, J = 2.0 Hz, 1H)	6.00 (d, J = 1.4 Hz, 1H)	6.01 (s, 1H)
37	2.08 (s, 3H)	2.06 (s, 3H)	2.06 (s, 3H)
38	3.73 (s, 3H)	3.71 (s, 3H)	3.71 (s, 3H)
40	5.81 (d, J = 15.2 Hz, 1H)	5.81 (d, J = 15.6 Hz, 1H)	5.80 (d, J = 15.1 Hz, 1H)
41	7.28 (dd, J = 15.4, 10.2 Hz, 1H)	7.28 (m, 1H)	7.28 (m, 1H)
42	6.18 (m, 1H)	6.18 (m, 1H)	6.18 (m, 1H)
43	6.17 (m, 1H)	6.17 (m, 1H)	6.17 (m, 1H)
44	2.16 (m, 2H)	2.16 (m, 2H)	2.16 (m, 2H)
45	1.47 (sext, J = 7.4 Hz, 2H)	1.47 (sext, J = 7.3 Hz, 2H)	1.47 (sext, J = 7.6 Hz, 2H)
46	0.92 (t, J = 7.4 Hz, 3H)	0.93 (t, J = 7.3 Hz, 3H)	0.93 (t, J = 7.3 Hz, 3H)
47	not mentioned	3.67 (s, 3H)	3.67 (s, 3H)

(a) Taken from Mag. Res. Chem. 1991, 29, 366-374.

(b) Measured under similar concentrations (2 mg/235 µL $CDCl_3$).

Table 2.2 Comparison of ^{13}C NMR Chemical Shift for Bryostatin 1

Carbon	Natural[a]	Natural[b]	Synthetic[b]
C_1	172.2	172.2	172.2
C_2	42.2	42.3	42.3
C_3	68.4	68.4	68.4
C_4	39.8	39.9	39.8
C_5	65.7	65.7	65.7
C_6	33.3	33.3	33.3
C_7	72.9	72.8	72.8
C_8	41.0	41.0	41.0
C_9	101.8	101.8	101.8
C_{10}	41.9	41.9	41.9
C_{11}	71.5	71.5	71.5
C_{12}	44.1	44.2	44.1
C_{13}	156.8	156.7	156.7
C_{14}	36.4	36.4	36.4
C_{15}	79.1	79.1	79.1
C_{16}	129.5	129.4	129.4
C_{17}	139.1	139.2	139.2
C_{18}	44.8	44.9	44.9
C_{19}	99.0	99.0	99.0
C_{20}	74.0	74.1	74.0
C_{21}	151.9	151.9	152.0
C_{22}	31.3	31.3	31.3
C_{23}	64.7	64.7	64.7
C_{24}	35.9	35.9	35.9
C_{25}	73.6	73.7	73.6
C_{26}	70.1	70.2	70.2
C_{27}	19.7	19.8	19.8
C_{28}	16.8	16.8	16.8
C_{29}	21.0	21.1	21.1
C_{30}	114.2	114.3	114.3
C_{31}	166.7	166.7	166.7
C_{32}	24.6	24.6	24.6
C_{33}	19.7	19.7	19.8
C_{34}	119.5	119.5	119.6
C_{35}	167.0	167.0	167.0
C_{36}	171.0	170.9	170.9
C_{37}	21.1	21.2	21.1
C_{38}	51.0	51.1	51.1
C_{39}	165.5	165.5	165.6
C_{40}	118.6	118.6	118.6
C_{41}	146.3	146.3	146.4

Table 2.2 Continued

Carbon	Natural[a]	Natural[b]	Synthetic[b]
C_{42}	128.3	128.3	128.4
C_{43}	145.4	145.5	145.5
C_{44}	35.0	35.1	35.1
C_{45}	21.8	21.9	21.9
C_{46}	13.6	13.7	13.7
C_{47}	51.0	51.1	51.1

Table 2.3 .Comparison of ^{13}C DEPT Chemical Shifts for Bryostatin 1

Carbon	Natural[b]	Synthetic[b]
CH_3		
C_{47}	51.1	51.1
C_{38}	51.1	51.1
C_{32}	24.6	24.6
C_{37}	21.2	21.2
C_{29}	21.1	21.1
C_{33}	19.8	19.8
C_{27}	19.7	19.8
C_{28}	16.8	16.8
C_{46}	13.7	13.7
CH_2		
C_{12}	44.1	44.1
C_2	42.3	42.3
C_{10}	41.9	41.9
C_4	39.9	39.8
C_{14}	36.4	36.4
C_{24}	35.9	35.9
C_{44}	35.1	35.0
C_6	33.3	33.3
C_{22}	31.5	31.3
C_{45}	21.9	21.9
CH_1		
C_{41}	146.4	146.4
C_{43}	145.5	145.5
C_{17}	139.2	139.2
C_{16}	129.4	129.4
C_{42}	128.3	128.4
C_{34}	119.5	119.6
C_{40}	118.6	118.6
C_{30}	114.3	114.3
C_{15}	79.1	79.1
C_{20}	74.0	74.0
C_{25}	73.8	73.7
C_7	72.8	72.8
C_{11}	71.5	71.5
C_{26}	70.2	70.2
C_3	68.4	68.4
C_5	65.7	65.7
C_{23}	64.7	64.7
CH_0		
C_1	172.2	172.2

Table 2.3 .Continued

Carbon	Natural[b]	Synthetic[b]
C_8	41.0	41.0
C_9	101.8	101.8
C_{13}	156.7	156.7
C_{18}	44.9	44.9
C_{19}	99.0	99.0
C_{21}	151.9	152.0
C_{31}	166.7	166.7
C_{35}	167.0	167.0
C_{36}	170.9	170.9
C_{39}	165.5	165.6

References

1. Pettit, G. R.; Day, J. F.; Hartwell, J. L.; Wood, H. B., *Nature* **1970,** *227*, 962-963.

2. Pettit, G. R.; Herald, C. L.; Doubek, D. L.; Herald, D. L.; Arnold, E.; Clardy, J., *J Am. Chem. Soc.*, **1982,** *104*, 6846-6848.

3. Pettit, G. R.; Herald, D. L.; Gao, F.; Sengupta, D.; Herald, C. L., *J. Org. Chem.*, **1991,** *56*, 1337-1340.

4. (a) Pettit, G. R.; Herald, C. L.; Kamano, Y.; Gust, D.; Aoyagi, R. *J. Nat. Prod.* **1983**, *46*, 528-531. (b) Pettit, G. R.; Herald, C. L.; Kamano, Y. *J. Org. Chem.* **1983**, *48*, 5354-5356. (c) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tozawa, M. *J. Am. Chem. Soc.* **1984**, *106*, 6768-6771. (d) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tozawa, M. *Can. J. Chem.* **1985**, *63*, 1204-1208. (e) Pettit, G. R.; Kamano, Y.; Aoyagi, R.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Rudloe, J. J. *Tetrahedron* **1985**, *41*, 985-994. (f) Pettit, G. R.; Kamano, Y.; Herald, C. L. *J. Nat. Prod.* **1986**, *49*, 661-664. (g) Pettit, G. R.; Kamano, Y.; Herald, C. L. *J. Org. Chem.* **1987**, *52*, 2848-2854. (h) Pettit, G. R.; Leet, J. E.; Herald, C. L.; Kamano, Y.; Boettner, F. E.; Baczynskyj, L.; Nieman, R. A. *J. Org. Chem.* **1987**, *52*, 2854-2860. (i) Pettit, G. R.; Gao, F.; Sengupta, D.; Coll, J. C.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Van Camp, J. R.; Rudloe, J. J.; Nieman, R. A. *Tetrahedron* **1991**, *47*, 3601-3610. (j) Pettit, G. R.; Gao, F.; Blumberg, P. M.; Herald, C. L.; Coll, J. C.; Kamano, Y.; Lewin, N. E.; Schmidt, J. M.; Chapuis, J.-C. *J. Nat. Prod.* **1996**, *59*, 286-289. (k) Lopanik, N.; Gustafson, K. R.; Lindquist, N. *J. Nat. Prod.* **2004**, *67*, 1412-1414.

5. For further information, see: http://clinicaltrials.gov.

6. (a) Spitaler, M.; Utz, I.; Hilbe, W.; Hofmann, J.; Grunicke, H. H. *Biochem. Pharmacol.* **1998**, *56*, 861-869. (b) Elgie, A. W.; Sargent, J. M.; Alton, P.; Peters, G. J.; Noordhuis, P.; Williamson, C. J.; Taylor, C. G. *Leuk. Res.* **1998**, *22*, 373-378.

7. (a) Basu, A.; Lazo, J. S. *Cancer Res.* **1992**, *52*, 3119-3124. (a) Wang, S.; Guo, C.-Y.; Castillo, A.; Dent, P.; Grant, S. *Biochem. Pharmacol.* **1998**, *56*, 635-644. (b) Al-Katib, A. M.; Smith, M. R.; Kamanda, W. S.; Pettit, G. R.; Hamdan, M.; Mohamed, A. N.; Chelladurai, B.; Mohammad, R. M. *Clin. Cancer Res.* **1998**, *4*, 1305-1314. (c) McGown, A. T.; Jayson, G.; Pettit, G. R.; Haran, M. S.; Ward, T. H.; Crowther, D. *Br. J. Cancer* **1998**, *77*, 216-220. (d) Mohammad, R. M.; Wall, N. R.; Dutcher, J. A.; Al-Katib, A. M. *Clin. Cancer Res.* **2000**, *6*, 4950-4956. (e) Dowlati, A.; Lazarus, H. M.; Hartman, P.; Jacobberger, J. W.; Whitacre, C.; Gerson, S. L.; Ksenich, P.; Cooper, B. W.; Frisa, P. S.; Gottlieb, M.; Murgo, A. J.; Remick, S. C. *Clin. Cancer Res.* **2003**, *9*, 5929-5935.

8. (a) Berkow, R. L.; Kraft, A. S. *Biochem. Biophys. Res. Commun.* **1985**, *131*, 1109-1116. (b) Philip, P. A.; Rea, D.; Thavasu, P.; Carmichael, J.; Stuart, N. S.; Rockett, H.; Talbot, D. C.; Ganesan, T.; Pettit, G. R.; Balkwill, F.; et al. *J. Natl. Cancer Inst.* **1993**, *85*, 1812-1818. (c) Bosco, M. C.; Rottschafer, S.; Taylor, L. S.; Ortaldo, J. R.; Longo, D. L.; Espinoza-Delgado, I. *Blood* **1997**, *89*, 3402-3411. (d) Do, Y.; Hegde, V. L.; Nagarkatti, P. S.; Nagarkatti, M. *Cancer Res.* **2004**, *64*, 6756-6765.

9. (a) Sun, M.-K.; Alkon, D. L. *Eur. J. Pharmacol.* **2005**, *512*, 43-51. (b) Kuzirian, A. M.; Epstein, H. T.; Gagliardi, C. J.; Nelson, T. J.; Sakakibara, M.; Taylor, C.; Scioletti, A. B.; Alkon, D. L. *Biol. Bull.* **2006**, *210*, 201-214. (c) Sun, M.-K.; Alkon, D. L. *Eur. J. Pharmacol.* **2008**, *584*, 328-337.

10. (a) Etcheberrigaray, R.; Tan, M.; Dewachter, I.; Kuiperi, C.; van der Auwera, I.; Wera, S.; Qiao, L.; Bank, B.; Nelson, T. J.; Kozikowski, A. P.; van Leuven, F.; Alkon, D. L. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 11141-11146. (b) Sun, M.-K.; Alkon, D. L. *CNS Drug Rev.* **2006**, *12*, 1-8. 11. Sun, M.-K.; Hongpaisan, J.; Nelson, T. J.; Alkon, D. L. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 13620-13625.

12. Mehla, R.; Bivalkar-Mehla, S.; Zhang, R.; Handy, I.; Albrecht, H.; Giri, S.; Nagarkatti, P.; Nagarkatti, M.; Chauhan, A., *PLoS One*. **2010**, *5*, e11160.
13. (a) Berkow, R. L.; Kraft, A. S. *Biochem. Biophys. Res. Commun.* **1985**, *131*, 1109-1116. (b) Kraft, A. S.; Smith, J. B.; Berkow, R. L. *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83*, 1334-1338. (c) Blumberg, P. M. *Cancer Res.* **1988**, *48*, 1-8.

14. Schaufelberger, D. E.; Koleck, M. P.; Beutler, J. A.; Vatakis, A. M.; Alvarado, A. B.; Andrews, P.; Marzo, L. V.; Muschik, G. M.; Roach, J.; Ross, J. T. *J. Nat. Prod.* **1991**, *54*, 1265-1270.

15. Mendola, D., *Biomol. Eng.*, **2003**, *11*, 1543-1542.

16. Sudek, S.; Lopanik, N. B.; Waggoner, L. E.; Hildebrand, M.; Anderson, C.; Liu, H.; Patel, A.; Sherman, D. H.; Haygood, M. G. *J. Nat. Prod.* **2007**, *70*, 67-74.

17. (a) Masamune, S. *Pure Appl. Chem.* **1988**, *60*, 1587-1596. (b) Blanchette, M. A.; Malamas, M. S.; Nantz, M. H.; Roberts, J. C.; Somfai, P.; Whritenour, D. C.; Masamune, S.; Kageyama, M.; Tamura, T. *J. Org. Chem.* **1989**, *54*, 2817-2825. (c) Kageyama, M.; Tamura, T.; Nantz, M. H.; Roberts, J. C.; Somfai, P.; Whritenour, D. C.; Masamune, S. *J. Am. Chem. Soc.* **1990**, *112*, 7407-7408.

18. (a) Evans, D. A.; Carter, P. H.; Carreira, E. M.; Prunet, J. A.; Charette, A. B.; Lautens, M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2354-2359. (b) Evans, D. A.; Carter, P. H.; Carreira, E. M.; Charette, A. B.; Prunet, J. A.; Lautens, M. *J. Am. Chem. Soc.* **1999**, *121*, 7540-7552.

19. (a) Ohmori, K.; Nishiyama, S.; Yamamura, S. *Tetrahedron Lett.* **1995**, *36*, 6519-6522. (b) Ohmori, K.; Suzuki, T.; Nishiyama, S.; Yamamura, S. *Tetrahedron Lett.* **1995**, *36*, 6515-6518. (c) Obitsu, T.; Ohmori, K.; Ogawa, Y.; Hosomi, H.; Ohba, S.; Nishiyama, S.; Yamamura, S. *Tetrahedron Lett.* **1998**, *39*, 7349-7352. (d) Ohmori, K.; Ogawa, Y.; Obitsu, T.; Ishikawa, Y.; Nishiyama, S.; Yamamura, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 2290-2294.

20. (a) Trost, B. M.; Yang, H.; Thiel, O. R.; Frontier, A. J.; Brindle, C. S. *J. Am. Chem. Soc.* **2007**, *129*, 2206-2207. (b) Trost, B. M.; Dong, G. *Nature*, **2008**, *456*, 485-488.

21. (a) Hale, K. J.; Lennon, J. A.; Manaviazar, S.; Javaid, M. H.; Hobbs, C. J. *Tetrahedron Lett.* **1995**, *36*, 1359-1362. (b) Hale, K. J.; Hummersone, M. G.; Cai, J.; Manaviazar, S.; Bhatia, G. S.; Lennon, J. A.; Frigerio, M.; Delisser, V. M.; Chumnongsaksarp, A.; Jogiya, N.; Lemaitre, A. *Pure Appl. Chem.* **2000**, *72*, 1659-1670. (c) Hale, K. J.; Hummersone, M. G.; Bhatia, G. S. *Org. Lett.* **2000**, *2*, 2189-2192. (d) Hale, K. J.; Frigerio, M.; Manaviazar, S. *Org. Lett.* **2001**, *3*, 3791-3794. (e) Hale, K. J.; Frigerio, M.; Manaviazar, S. *Org. Lett.* **2003**, *5*, 503-505. (f) Manaviazar, S.; Frigerio, M.; Bhatia, G. S.; Hummersone, M. G.; Aliev, A. E.; Hale, K. J. *Org. Lett.* **2006**, *8*, 4477-4480.

22. (a) Hoffmann, R. W.; Stiasny, H. C. *Tetrahedron Lett.* **1995**, *36*, 4595-4598. (b) Lampe, T. F. J.; Hoffmann, H. M. R. *Tetrahedron Lett.* **1996**, *37*, 7695-7698. (c) Weiss, J. M.; Hoffmann, H. M. R. *Tetrahedron Asymmetry* **1997**, *8*, 3913-3920. (d) Kalesse, M.; Eh, M. *Tetrahedron Lett.* **1996**, *37*, 1767-1770. (e) Kiyooka, S.-i.; Maeda, H. *Tetrahedron Asymmetry* **1997**, *8*, 3371-3374. (f) De Brabander, J.; Vanhessche, K.; Vandewalle, M. *Tetrahedron Lett.* **1991**, *32*, 2821-2824. (g) De Brabander, J.; Vandewalle, M. *Synthesis* **1994**, 855-865. (h) De Bradander, J.; Vandewalle, M. *Synlett* **1994**, 231-232. (i) De Brabander, J.; Vandewalle, M. *Pure Appl. Chem.* **1996**, *68*, 715-718. j) De Brabander, J.; Kulkarni, B. A.; Garcia-Lopez, R.; Vandewalle, M. *Tetrahedron Asymmetry* **1997**, *8*, 1721-1724. (k) Baxter, J.; Mata, E. G.; Thomas, E. J. *Tetrahedron* **1998**, *54*, 14359-14376. (l) Gracia, J.; Thomas, E. J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2865-2872. (m) Maguire, R. J.; Munt, S. P.; Thomas, E. J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2853-2864. (n) Almendros, P.; Rae, A.; Thomas, E. J. *Tetrahedron Lett.* **2000**, *41*, 9565-9568. (o) Lopez-Pelegrin, J. A.; Wentworth, P., Jr.; Sieber, F.; Metz, W. A.; Janda, K. D. *J. Org. Chem.* **2000**, *65*, 8527-8531. (p) Vakalopoulos, A.; Lampe, T. F. J.; Hoffmann, H. M. R. *Org. Lett.* **2001**, *3*, 929-932. (q) Yadav, J. S.; Bandyopadhyay, A.; Kunwar, A. C. *Tetrahedron Lett.* **2001**, *42*, 4907-4911. (r) O'Brien, M.; Taylor, N. H.; Thomas, E. J. *Tetrahedron Lett.* **2002**, *43*, 5491-5494. (s) Ball, M.; Baron, A.; Bradshaw, B.; Omori, H.; MacCormick, S.; Thomas, E. J. *Tetrahedron Lett.* **2004**, *45*, 8737-8740. (t) Voight, E. A.; Seradj, H.; Roethle, P. A.; Burke, S. D. *Org. Lett.* **2004**, *6*, 4045-4048. (u) Voight, E. A.; Roethle, P. A.; Burke, S. D. *J. Org. Chem.* **2004**, *69*, 4534-4537. (v) Cho, C.-W.; Krische, M. J. *Org. Lett.* **2006**, *8*, 891-894. (w) Allen, J. V.; Green, A. P.; Hardy, S.; Heron, N. M.; Lee, A. T. L.; Thomas, E. J. *Tetrahedron Lett.* **2008**, *49*, 6352-6355. (x)

Green, A. P.; Hardy, S.; Thomas, E. J. *Synlett* **2008**, 2103-2106. (y) Lu, Y.; Krische, M. J. *Org. Lett.* **2009**, *11*, 3108-3111.

23. (a) Hale, K. J.; Hummersome, M. G.; Manaviazar, S.; Frigerio, M. *Nat. Prod. Rep.* **2002**, *19*, 413–453. (b) Hale, K. J.; Manaviazar, S. *Chem. Asian J.* **2010**, *5*, 704-754.

24. Trost, B. M.; Dong, G., *J. Am. Chem. Soc.* **2010**, *132*, 16403-16416.

25. Keck, G. E.; Covel, J. A.; Schiff, T.; Yu, T. *Org. Lett.* **2002**, *4*, 1189-1192.

26. Keck, G. E.; Kraft, M. B.; Truong, A. P.; Li, W.; Sanchez, C. C.; Kedei, N.; Lewin, N.; Blumberg, P. M. *J. Am. Chem. Soc.* **2008**, *130*, 6660-6661.

27. Keck, G. E.; Li, W.; Kraft, M. B.; Kedei, N.; Lewin, N. E.; Blumberg, P. M. *Org. Lett*. **2009**, *11*, 2277-2280.

28. (a) Keck, G. E.; Poudel, Y. B.; Welch, D. S.; Kraft, M. B.; Truong, A. P.; Stephens, J. C.; Kedei, N.; Lewin, N. E.; Blumberg, P. M. *Org. Lett.* **2009**, *11*, 593-596.

29. Keck, G. E.; Poudel, Y. B.; Rudra, A.; Stephens, J. C.; Kedei, N.; E. Lewin, N. E.; Peach, M. L.; Blumberg, P. M. *Angew. Chem. Int. Ed.* **2010**, *49*, 4580-4584.

30. Wender, P. A.; DeChristopher, B. A.; Schrier, A. J. *J. Am. Chem. Soc.* **2008**, *130*, 6658-6659.

31. Keck, G. E.; Welch, D. S.; Vivian, P. K. *Org. Lett*. **2006**, *8*, 3667-3670.

32. (a) Keck, G. E.; Tarbet, K. H.; Geraci, L. S. *J. Am. Chem. Soc.* **1993**, *115*, 8467-8468. (b) Keck, G. E.; Krishnamurthy, D. *Org. Synth.* **1998**, *75*,12-16.

33. Semmelhack, M. F.; Stauffer, R. D.; Yamashita, A. *J. Org. Chem.* **1977**, *42*, 3180-3188.

34. (a) Bunnelle, W. H.; Narayanan, B. A. *Tetrahedron Lett*. **1987**, *28*, 6261-6264. (b) Bunnelle, W. H.; Narayanan, B. A. *Org. Synth*. **1990**, *8*, 89-92.

35. Corey, E. J.; Shibata, S.; Bakshi, R., K. *J. Chem. Soc., Chem. Commun.* **1988**, *8*, 469-470.

36. Keck, G. E.;Giles, R. L.; Cee, V. J.; Wager, C. A.; Yu T.; Kraft, M. B., *J. Org. Chem.*, **2008**, *73*, 9675-9691.

37. (a) Evans, D. A.; Chapman, K. T. *Tetrahedron Lett.*, **1986**, *27*, 5939-5942. (b) Saksena. A, X.; Mangiaracina, P., *Tetrahedron Lett.* **1983**, *24*, 273-276.

38. (a) Luche, J. L. *J. Am. Chem. Soc.* **1978**, *100*, 2226-2227. (b) Luche, J. L.; Rodriguez-Hahn, L.; Crabbe, P. *J. Chem. Soc., Chem. Commun.* **1978**, 601-602.

39. Keck, G. E.; Welch, D. S.; Poudel, Y. B. *Tetrahedron Lett.* **2006**, *47*, 8267-8270.

40. Takai, K.; Kakiuchi, T.; Kataoka, Y.; Utimoto, K. *J. Org. Chem.* **1994**, *59*, 2668.

41. Iyer, K.; Rainier, J. D., *J. Am. Chem. Soc.* **2007**, *129*, 12604-12605.

42. Thomas J. Cummins, University of Utah, Unpublished results.

43. Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *J. Chem. Soc., Chem. Commun.* **1987**, 1625-1627.

44. Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K. S.; Kwong, H. L.; Morikawa, K.; Wang, Z. M. *J. Org. Chem*. **1992**, *57*, 2768-2771.

45. (a) Tanaka, K.; Ohta, Y.; Fuji, K. *Tetrahedron Lett.* **1993**, *34*, 4071-4074. (b) For the previous use of the reagent in bryostatin synthesis, see ref 18(a) and 19(d).

46. Lipschutz, B. H.; Harvey, D. F. *Synth. Commun*. **1982**, *14*, 267-277.

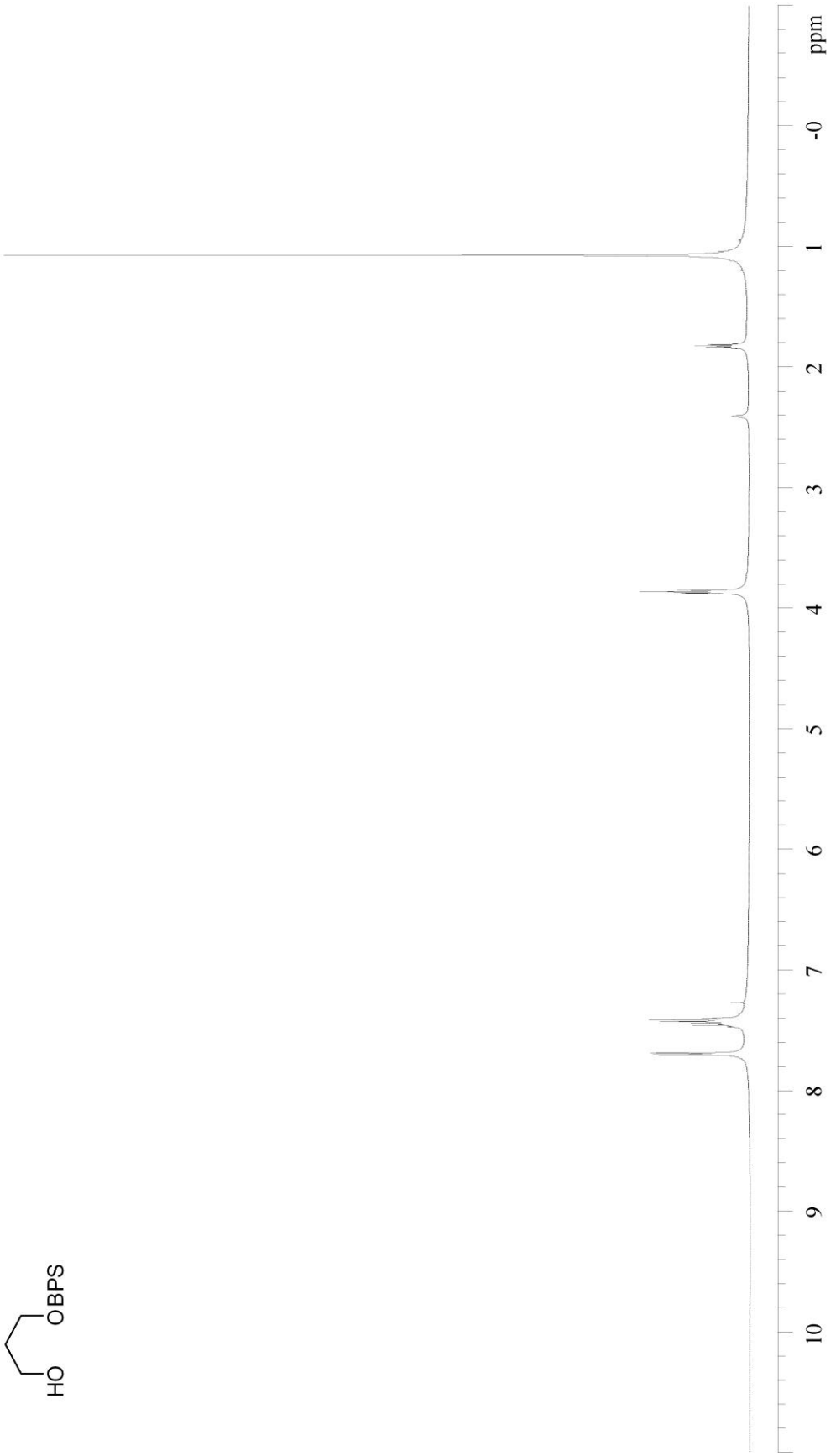
47. Schaufelberger, D. E.; Chmurny, G. N.; Koleck, M. P. *Mag. Res. Chem.* **1991**, *29*, 366-374.

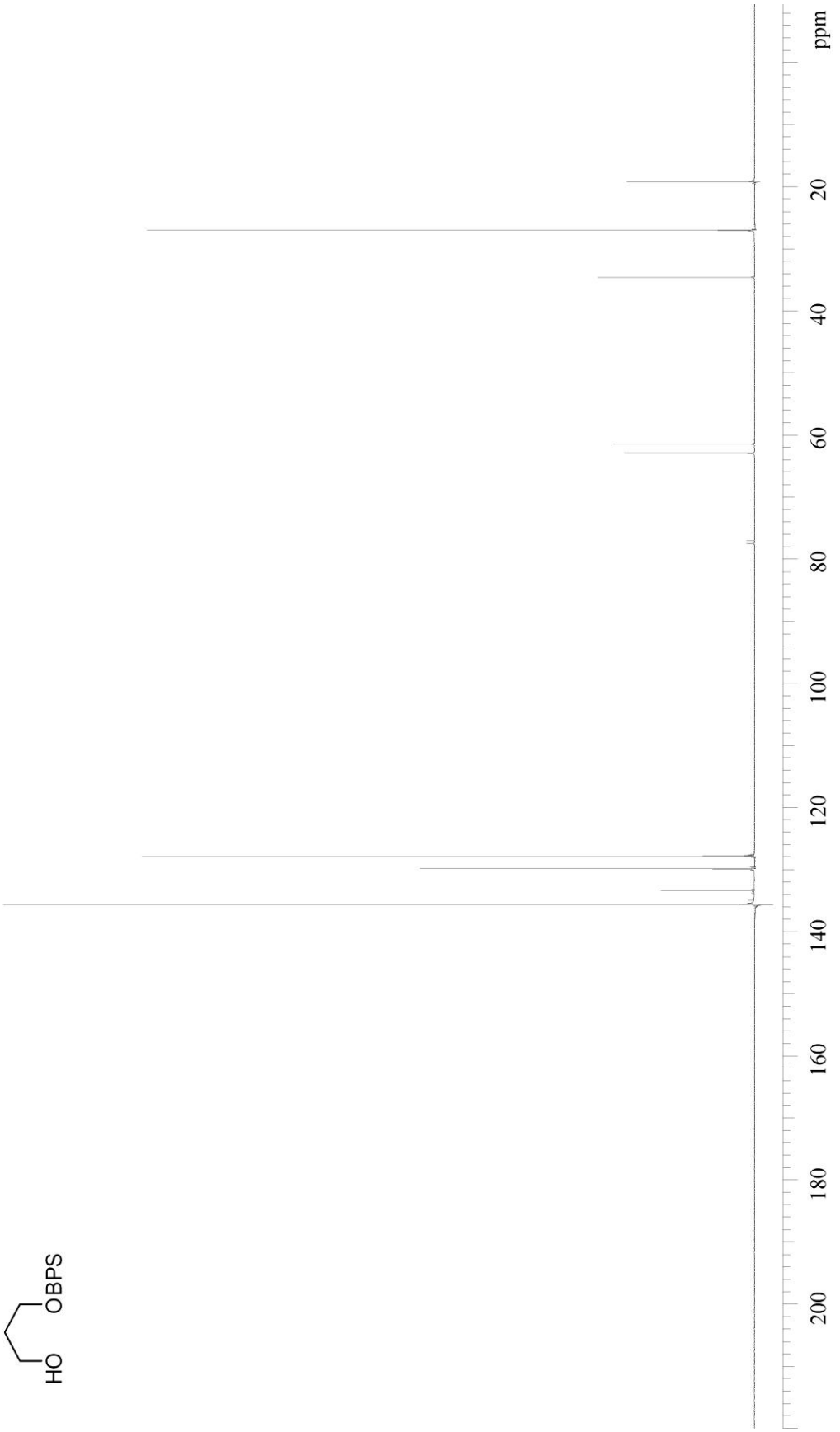
48. Armarego, W. L. F.; Perrin, D. D., *Purification of Laboratory Chemicals, Fourth Edition*. Butterworth-Heinemann: Oxford, **1997**.

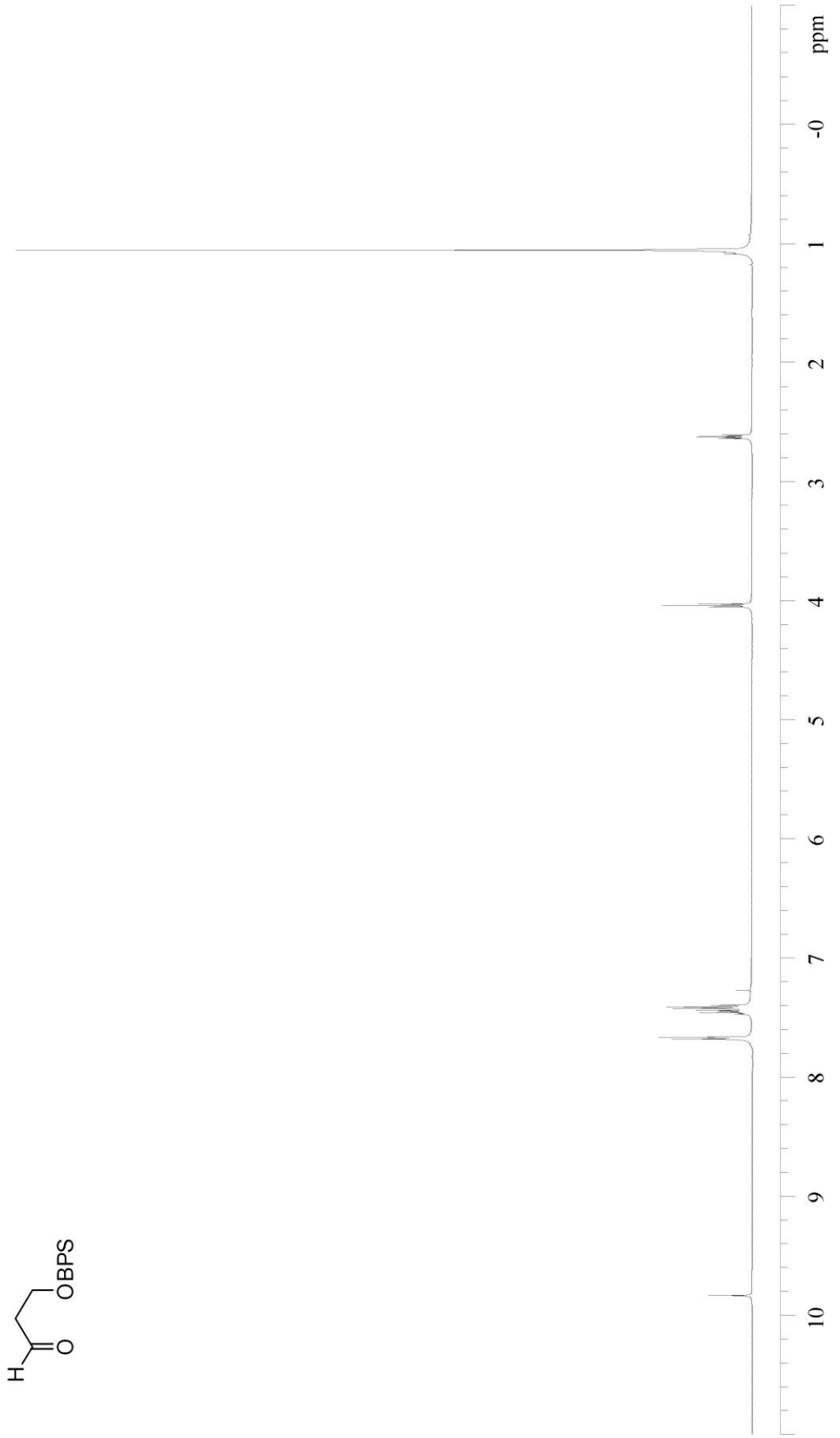
49. Watson, S. C.; Eastham, J. F. *J. Organomet. Chem.* **1967**, *9*, 165-168.

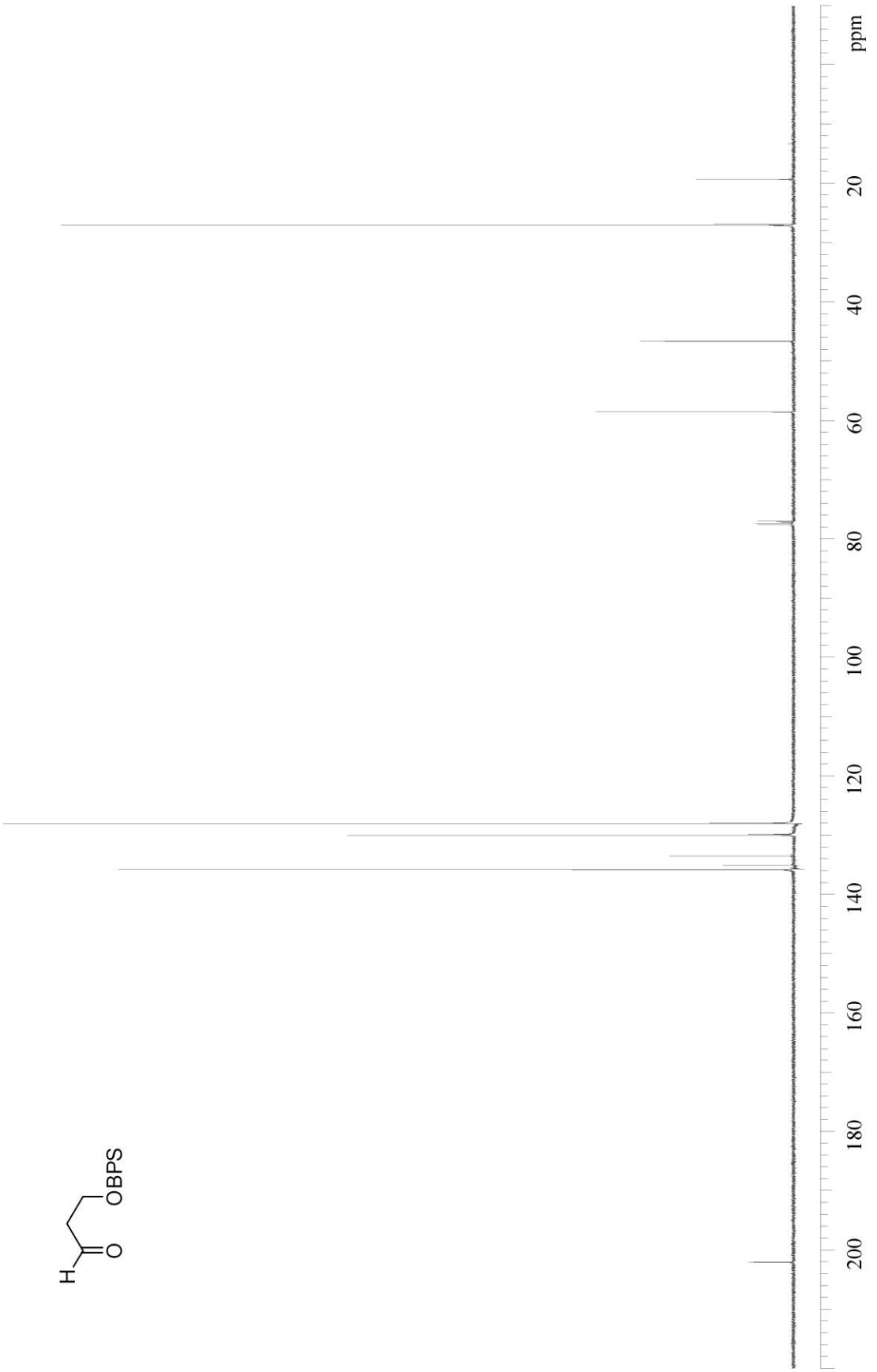
APPENDIX A

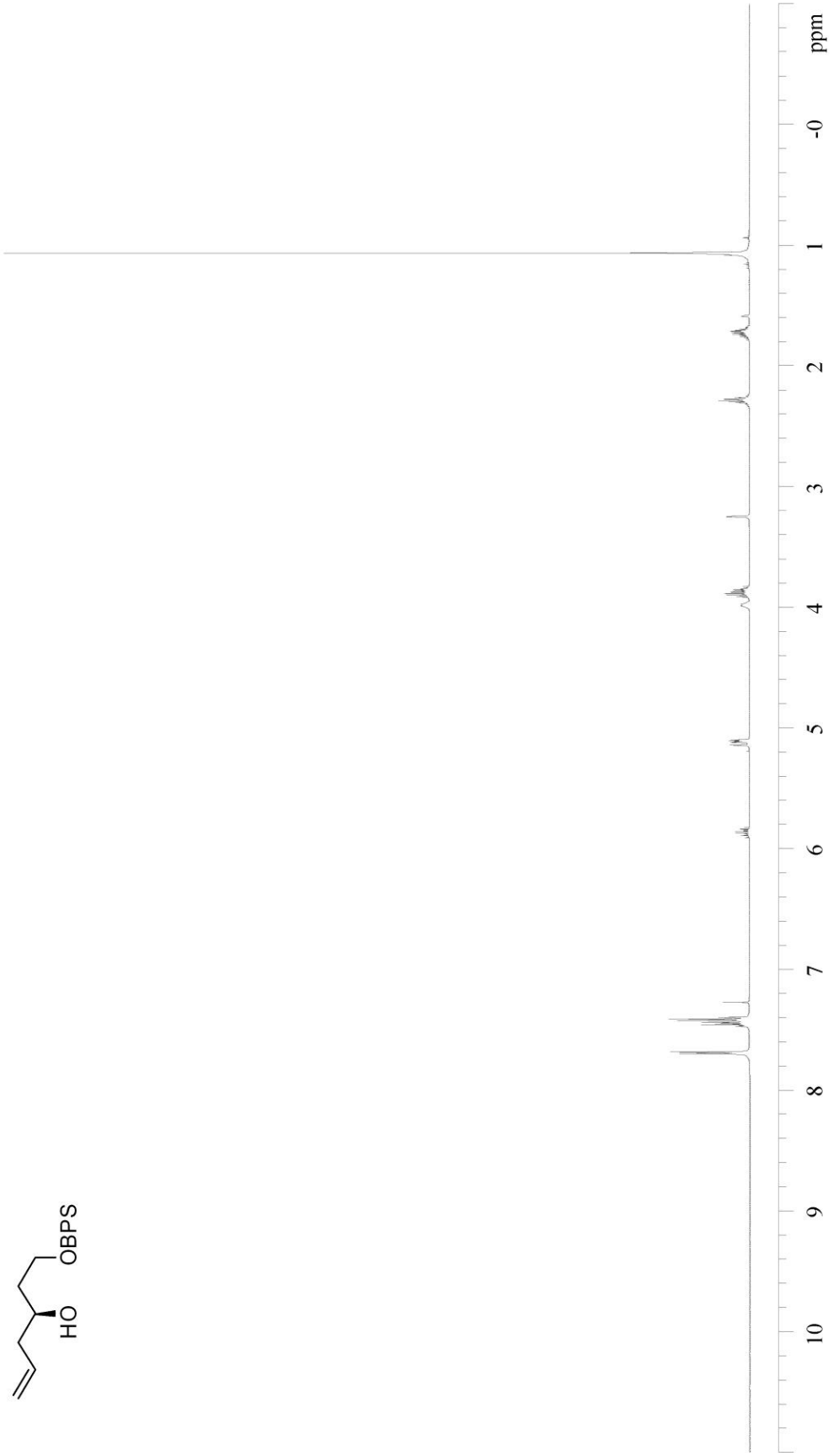
^{1}H, AND ^{13}C NMR SPECTRA FOR CHAPTER 1


HO
OBPS
10
9
8
7
6
5
4
3
2
1
-0
ppm

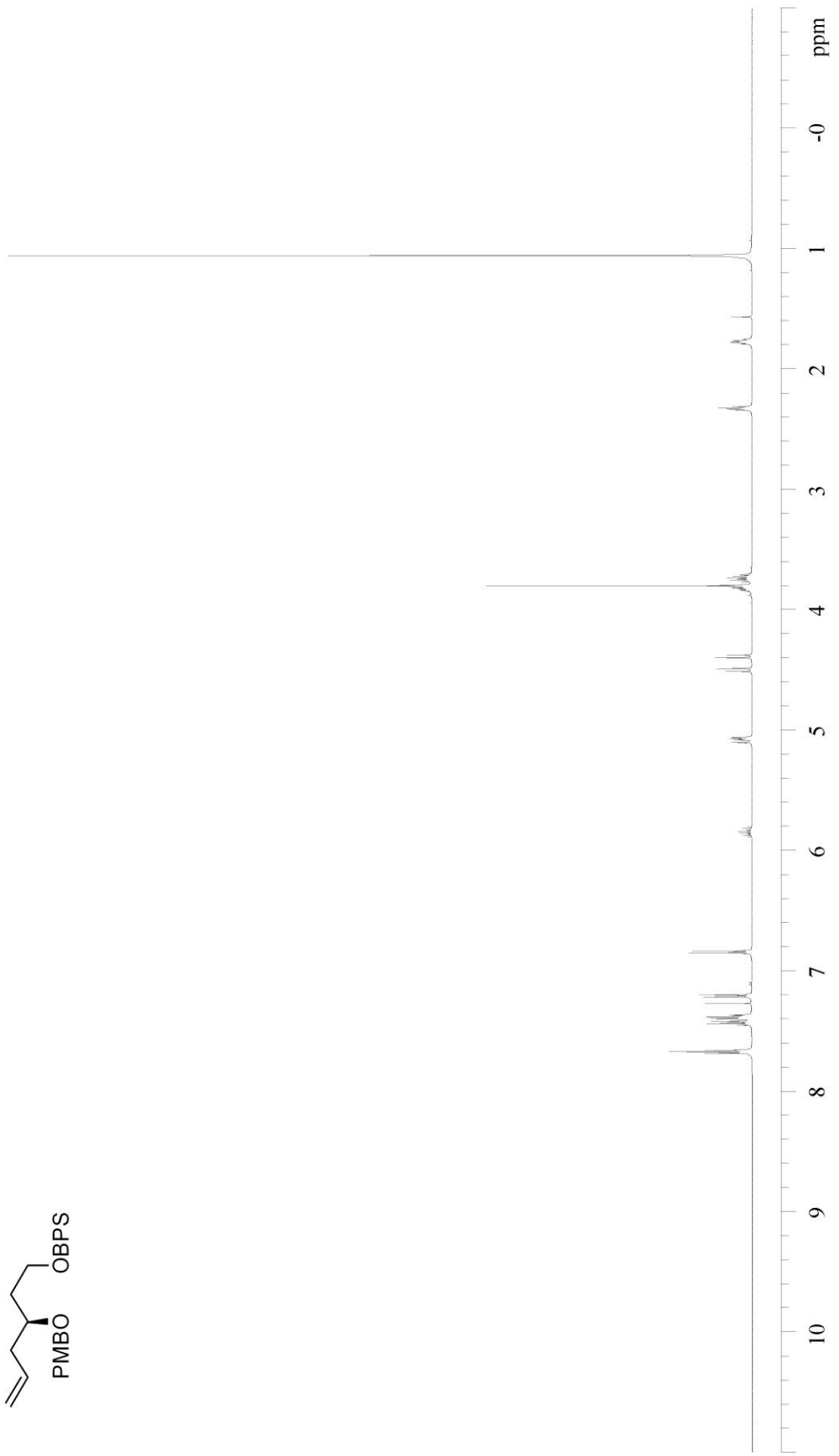

HO
OBPS
200
180
160
140
120
100
80
60
40
20
ppm


OBPS
O
H
10
9
8
7
6
5
4
3
2
1
-0
ppm

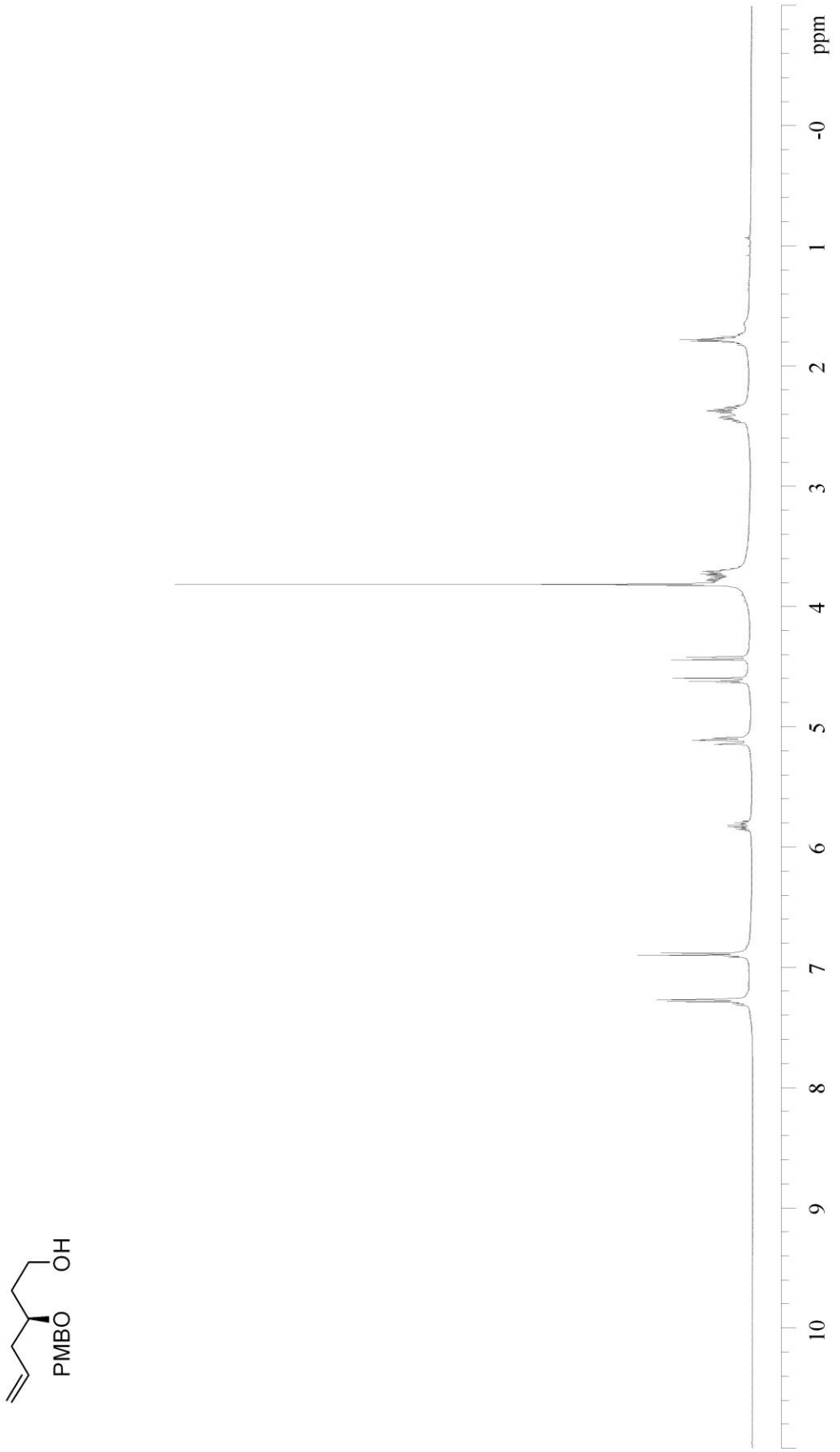

OBPS
O
H
ppm
200
180
160
140
120
100
80
60
40
20


HO
OBPS
10
9
8
7
6
5
4
3
2
1
-0
ppm

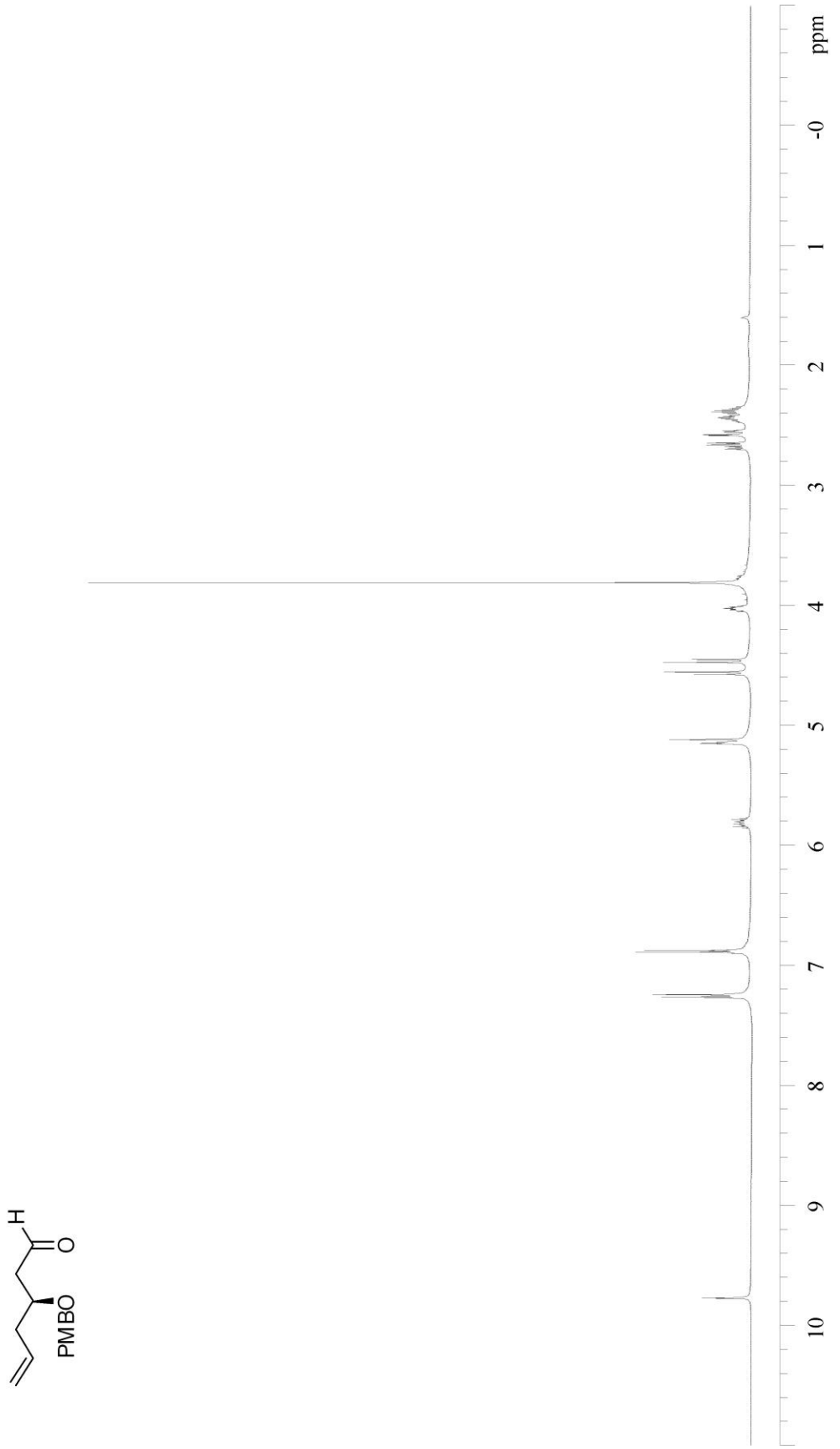

OBPS
HO
200
180
160
140
120
100
80
60
40
20
ppm

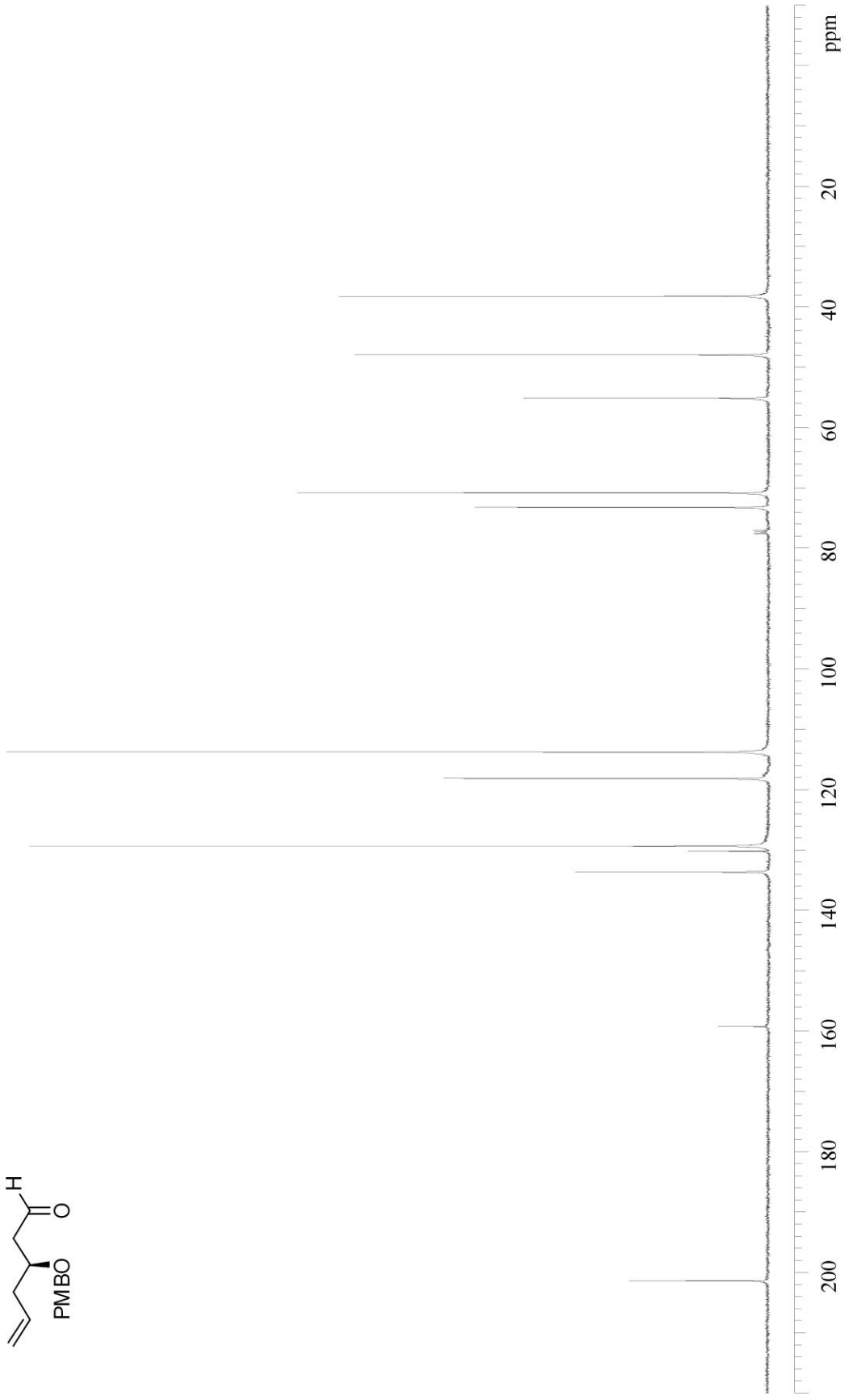

PMBO
OBPS
10
9
8
7
6
5
4
3
2
1
-0
ppm

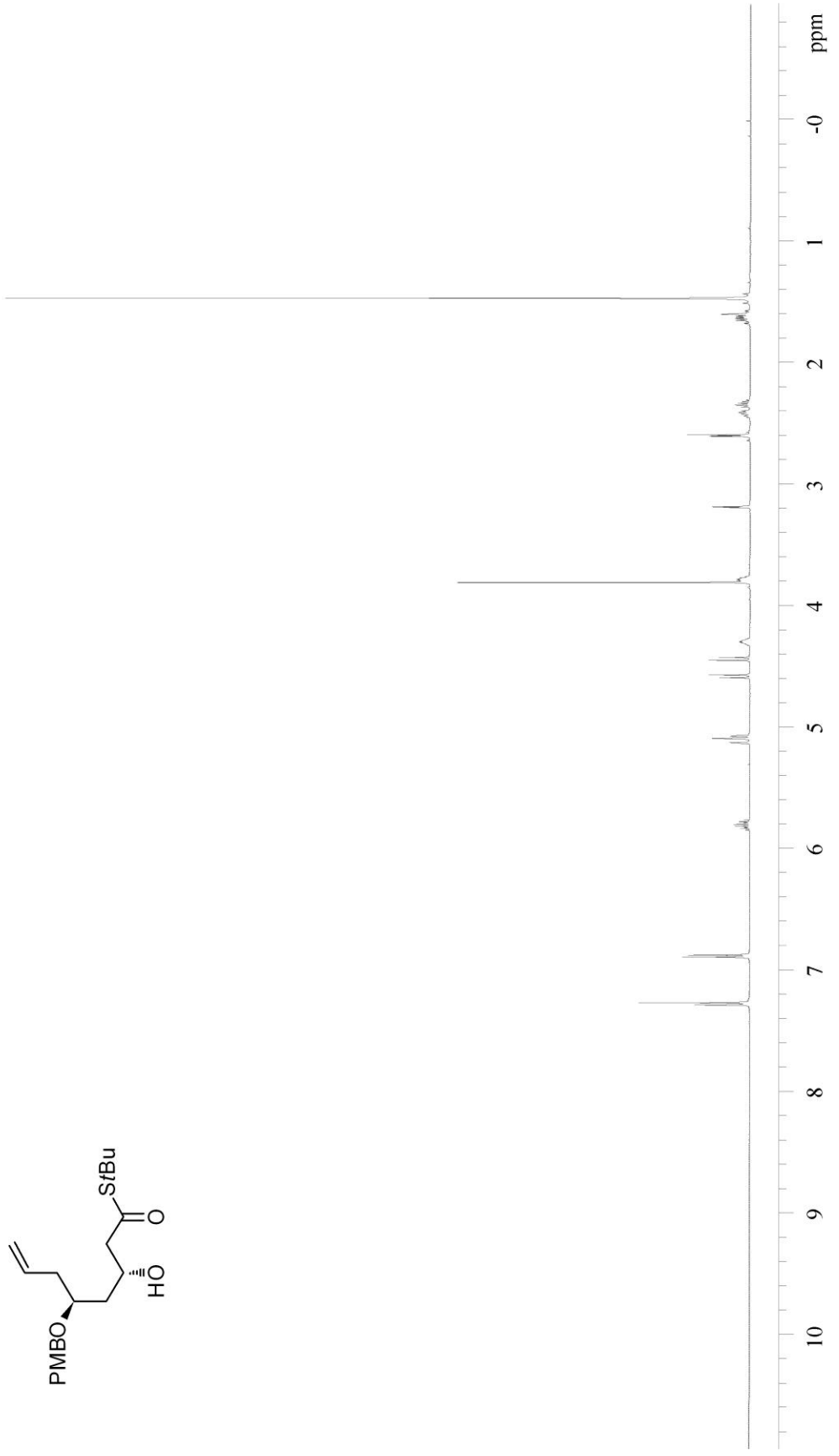

PMBO
OBPS
200
180
160
140
120
100
80
60
40
20
ppm

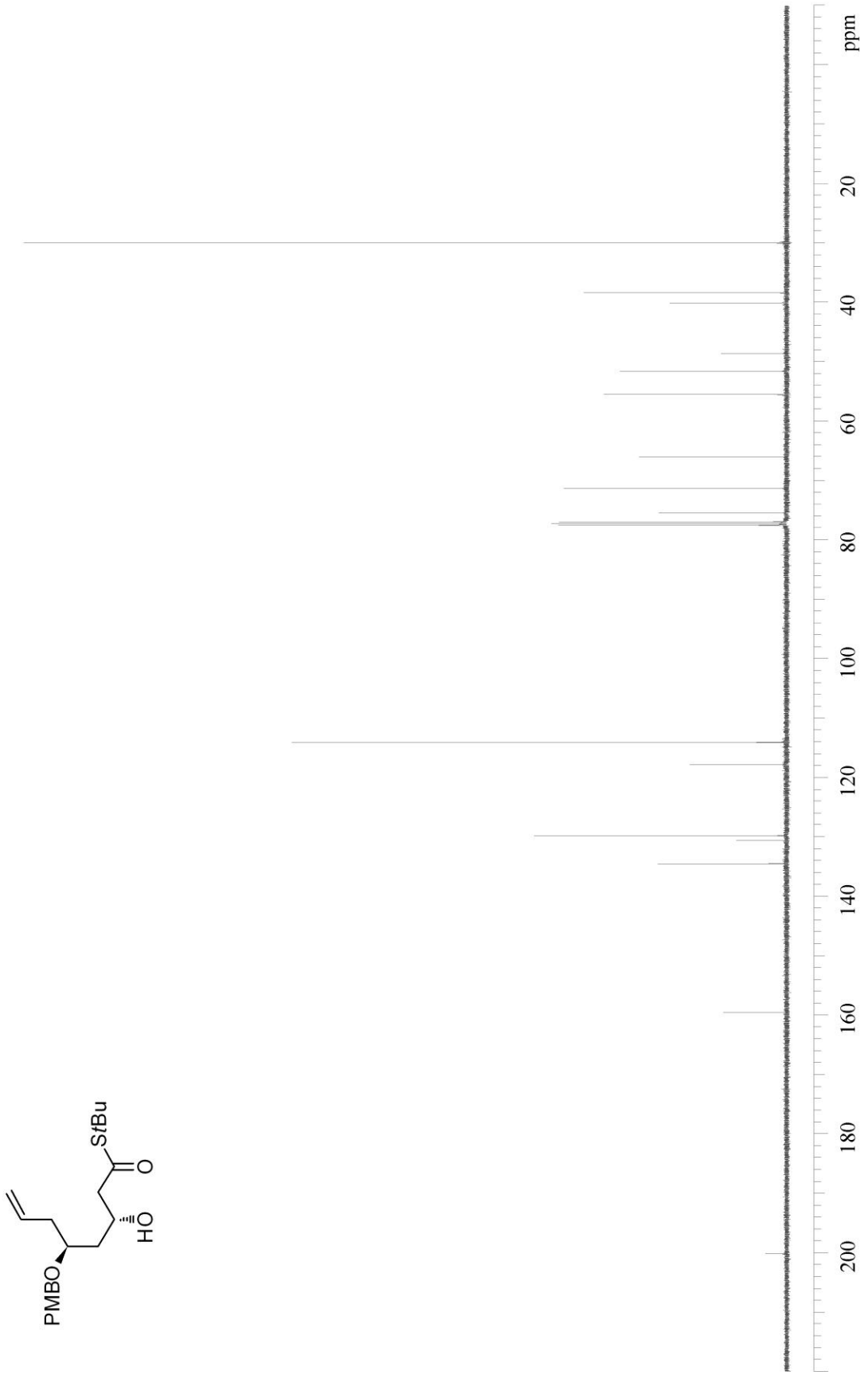

PMBO
OH
ppm
10
9
8
7
6
5
4
3
2
1
-0

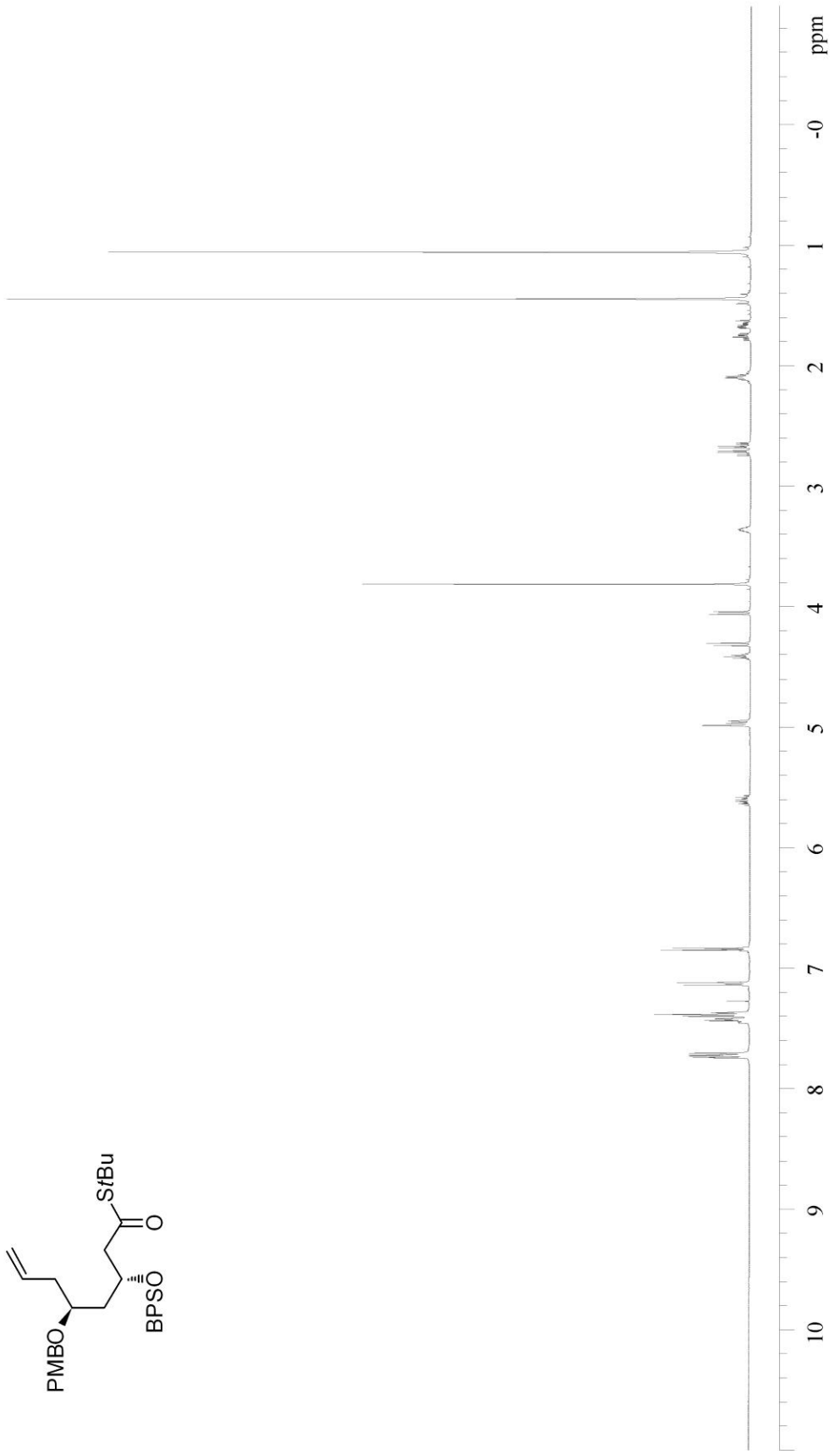

PMBO
OH
ppm
200
180
160
140
120
100
80
60
40
20

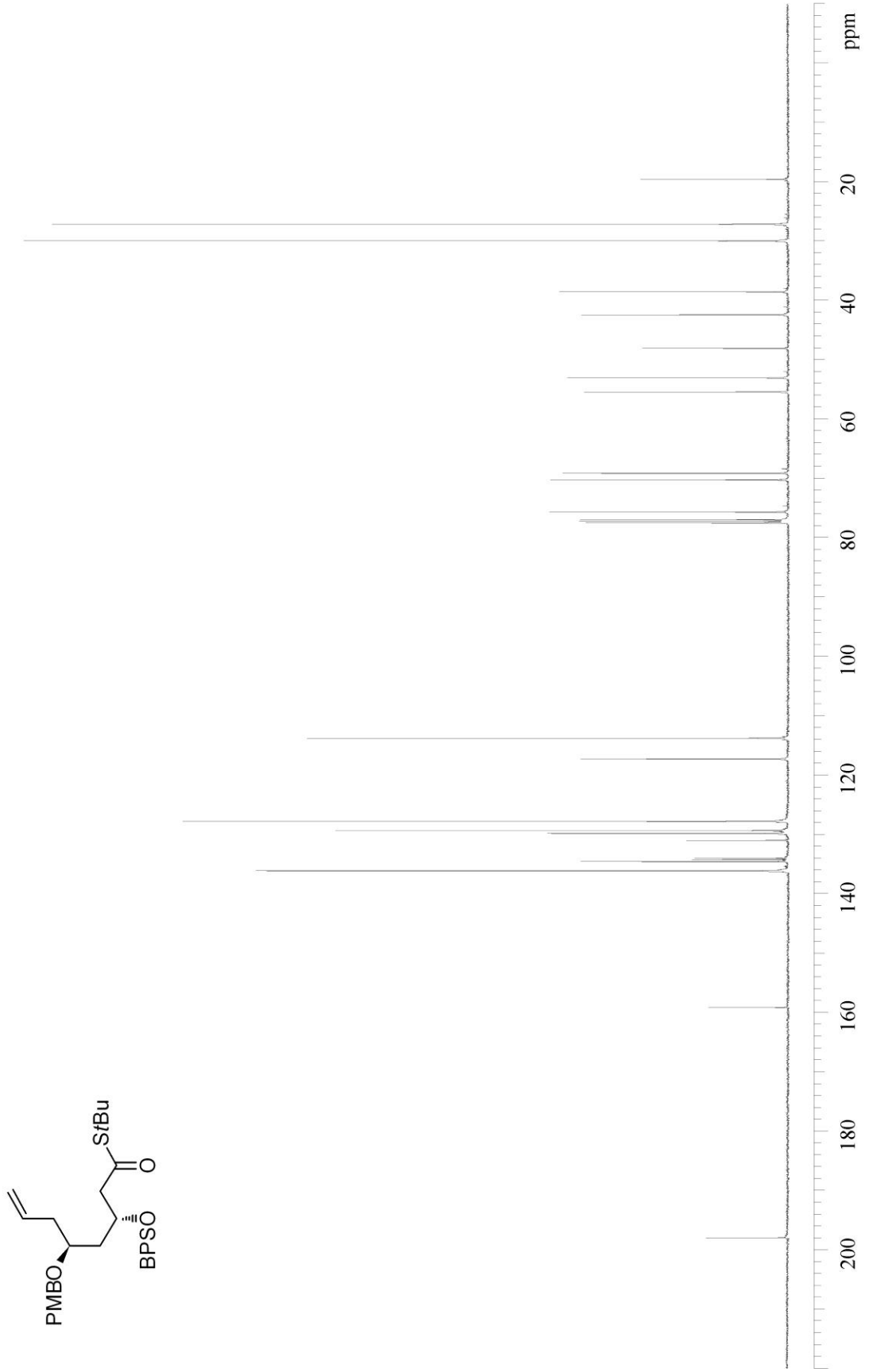

PMBO
O
H
10
9
8
7
6
5
4
3
2
1
-0
ppm

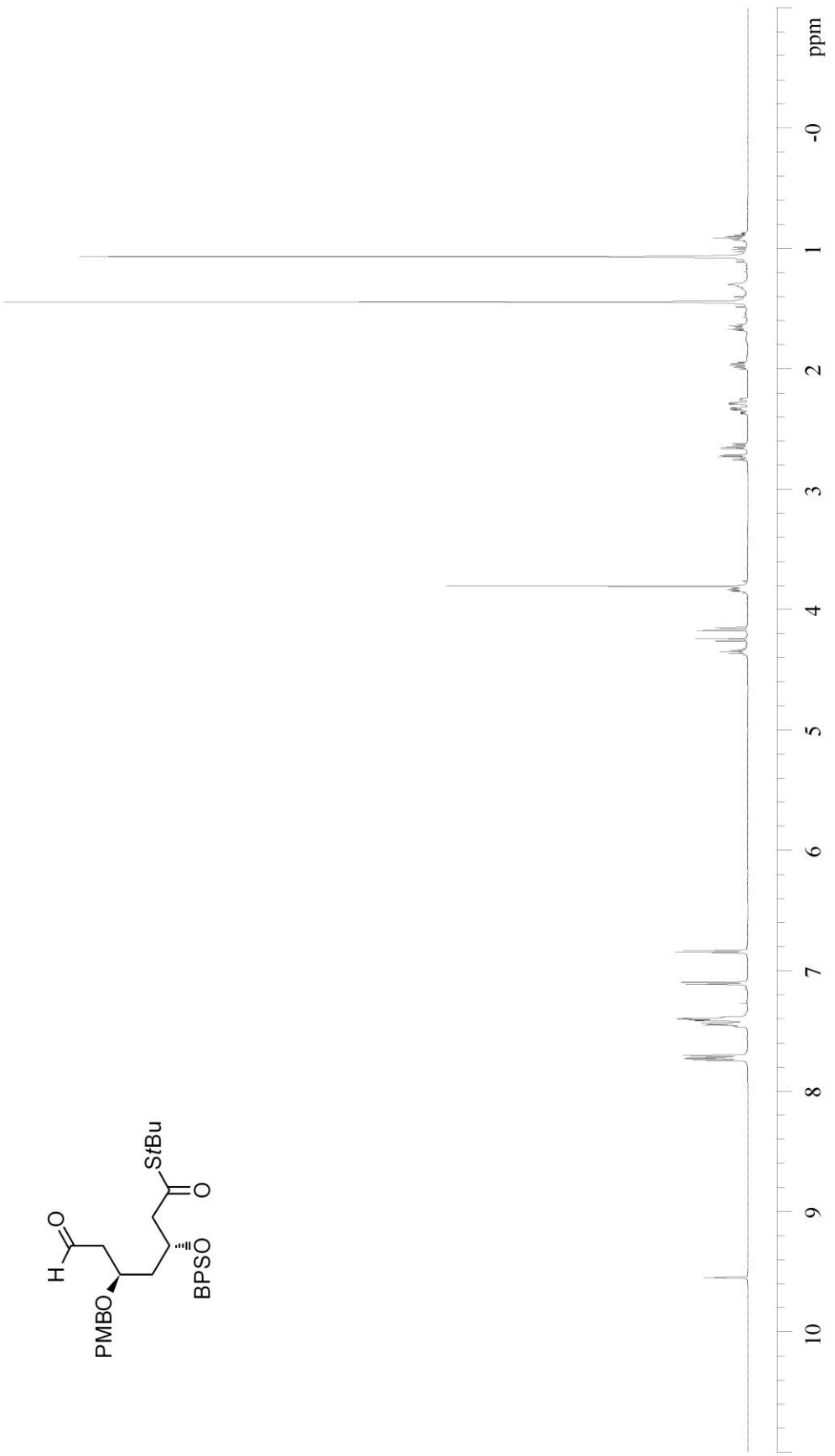

PMBO
O
H
200
180
160
140
120
100
80
60
40
20
ppm

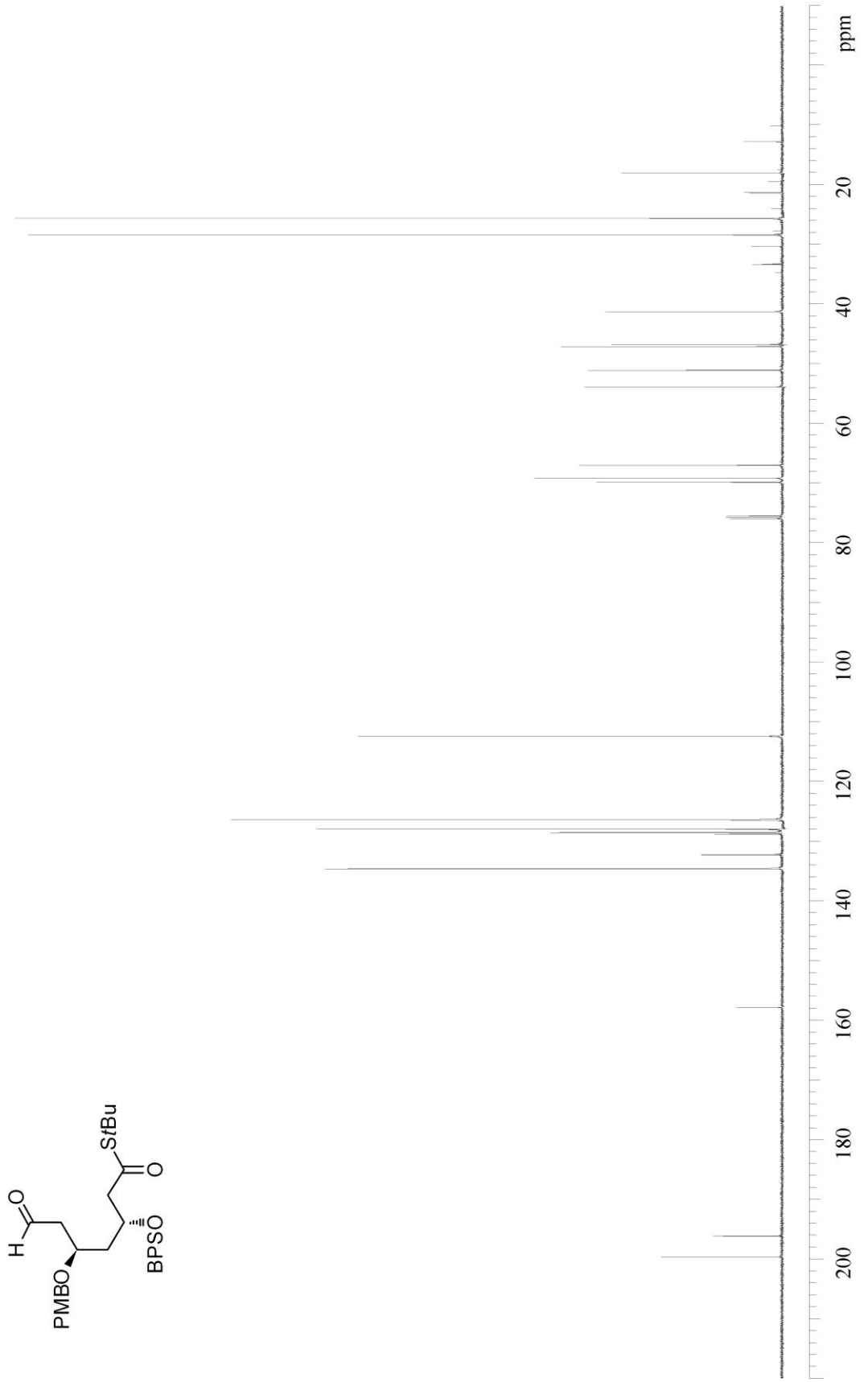

PMBO
HO
O
StBu
10
9
8
7
6
5
4
3
2
1
-0
ppm

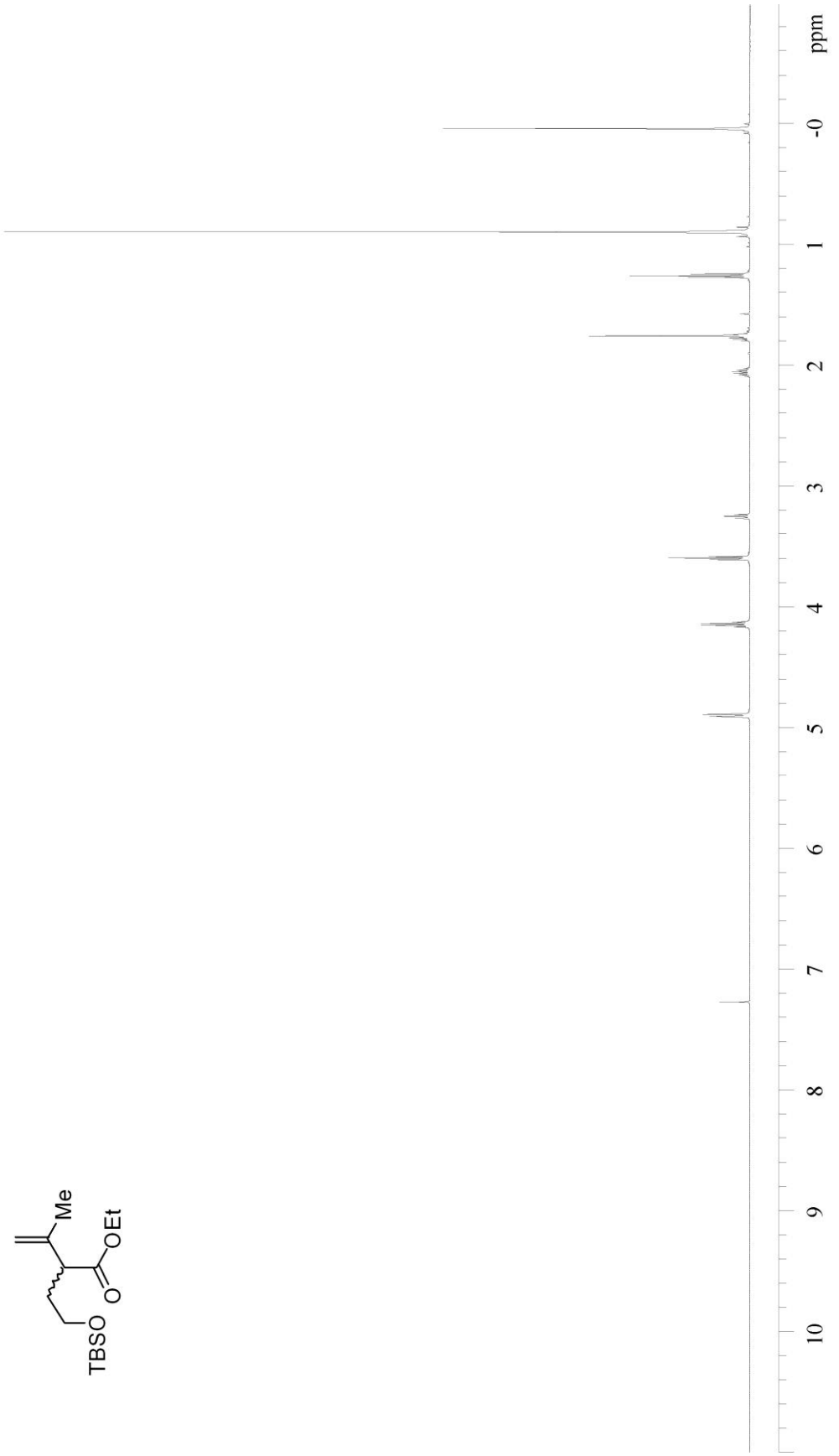

PMBO
HO
O
StBu
200
180
160
140
120
100
80
60
40
20
ppm

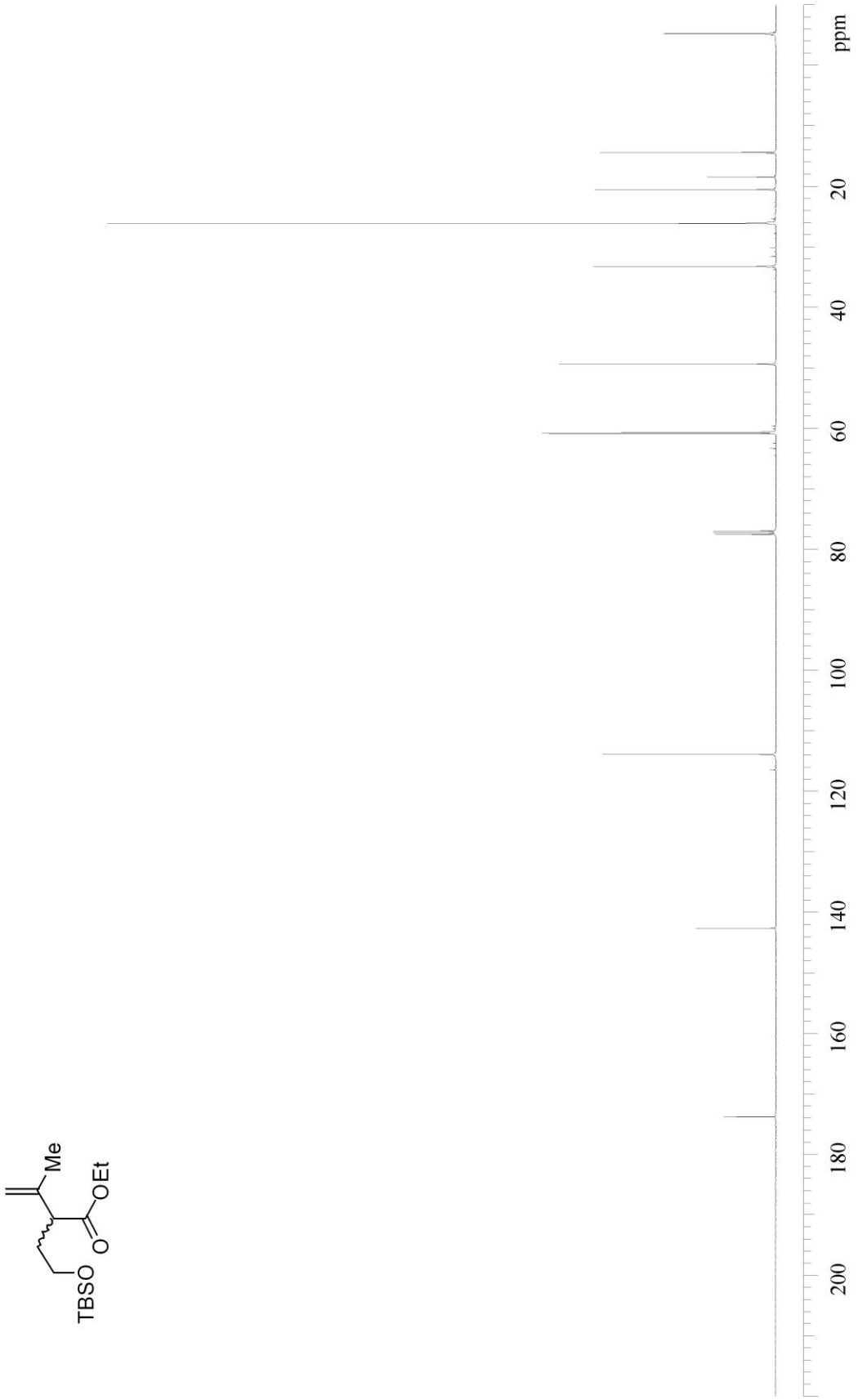

PMBO
BPSO
O
StBu
10
9
8
7
6
5
4
3
2
1
-0
ppm

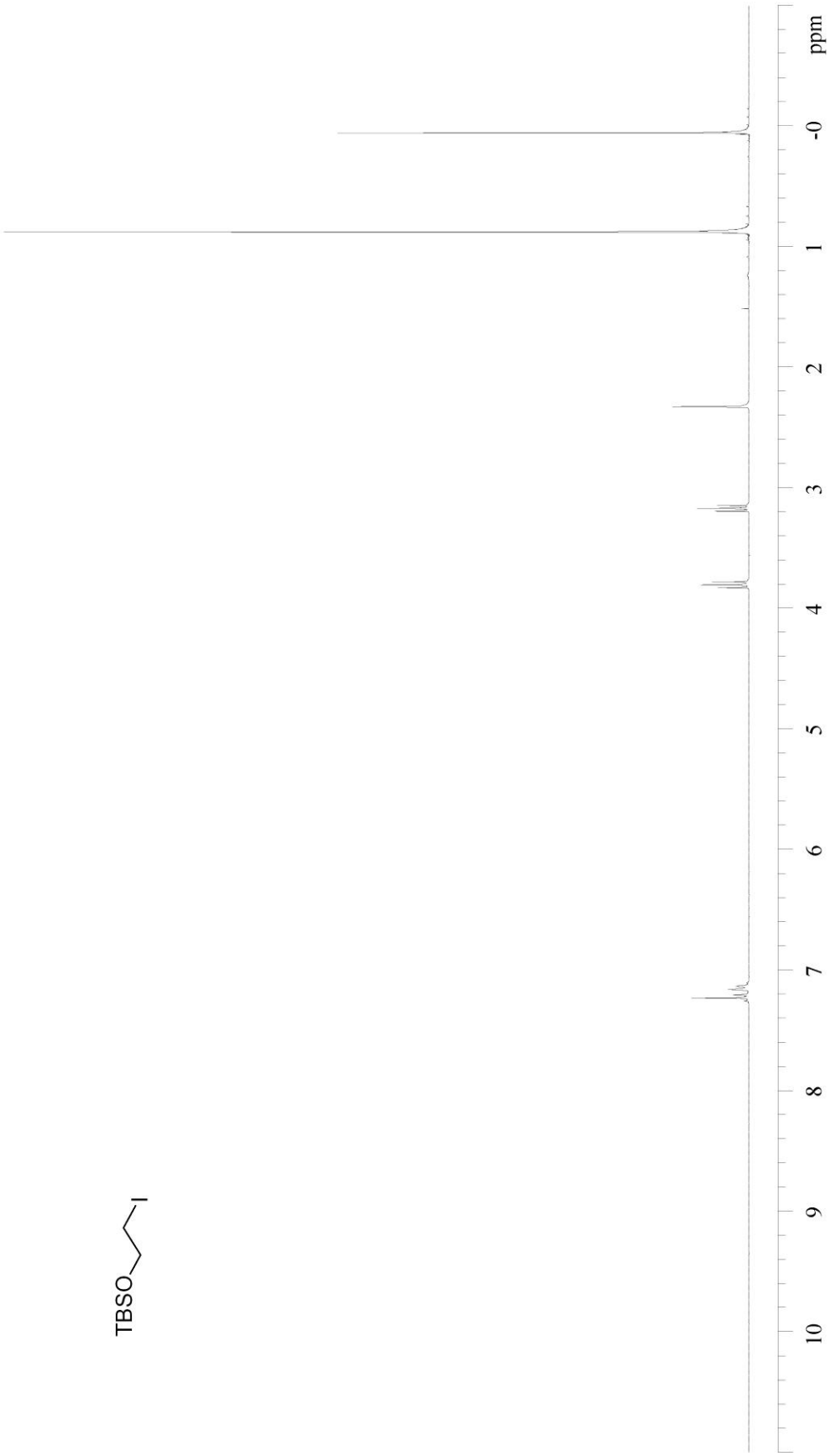

PMBO
BPSO
O
StBu
ppm
200
180
160
140
120
100
80
60
40
20

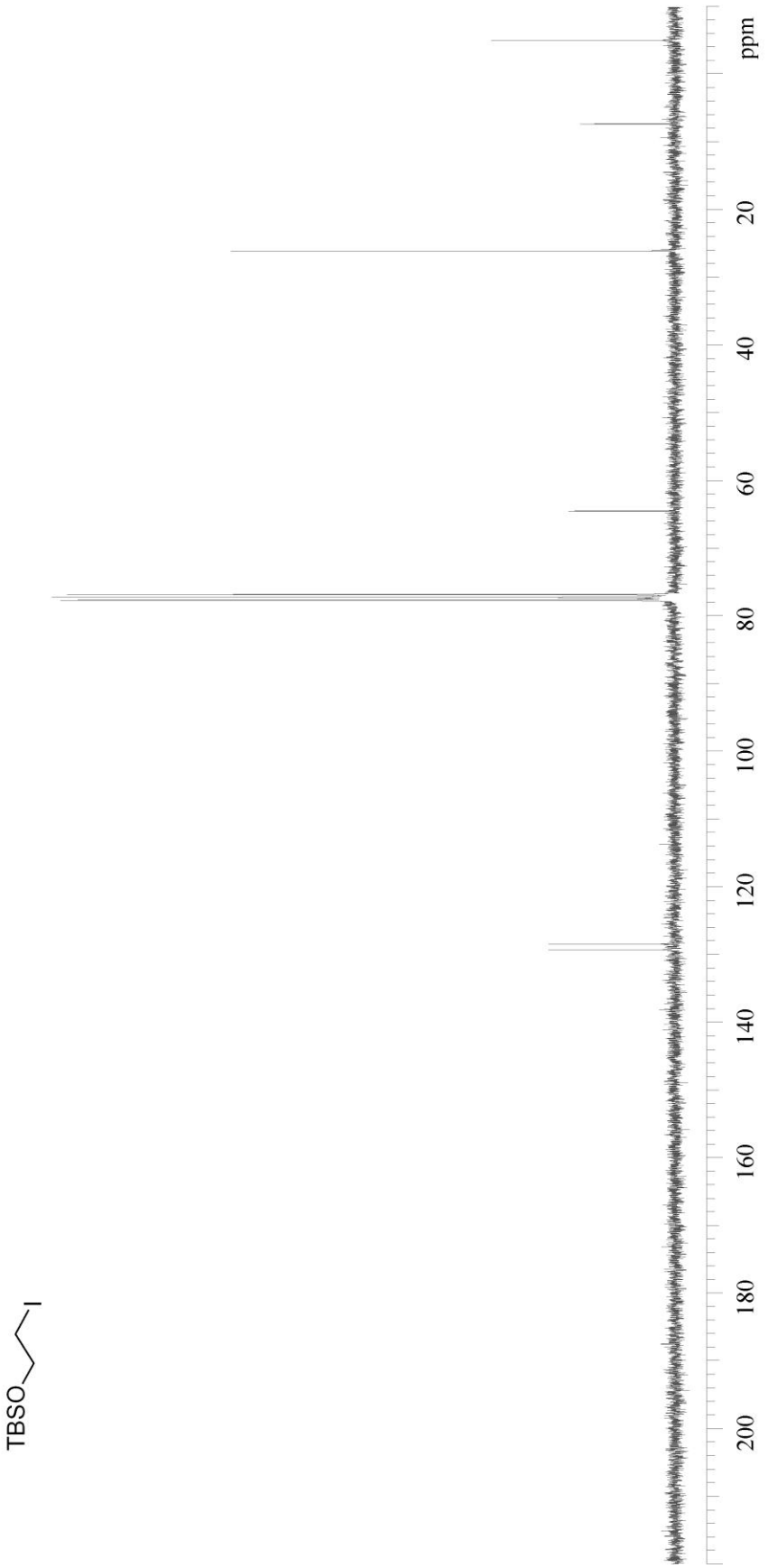

H
O
PMBO
BPSO
O
S*t*Bu
10
9
8
7
6
5
4
3
2
1
-0
ppm

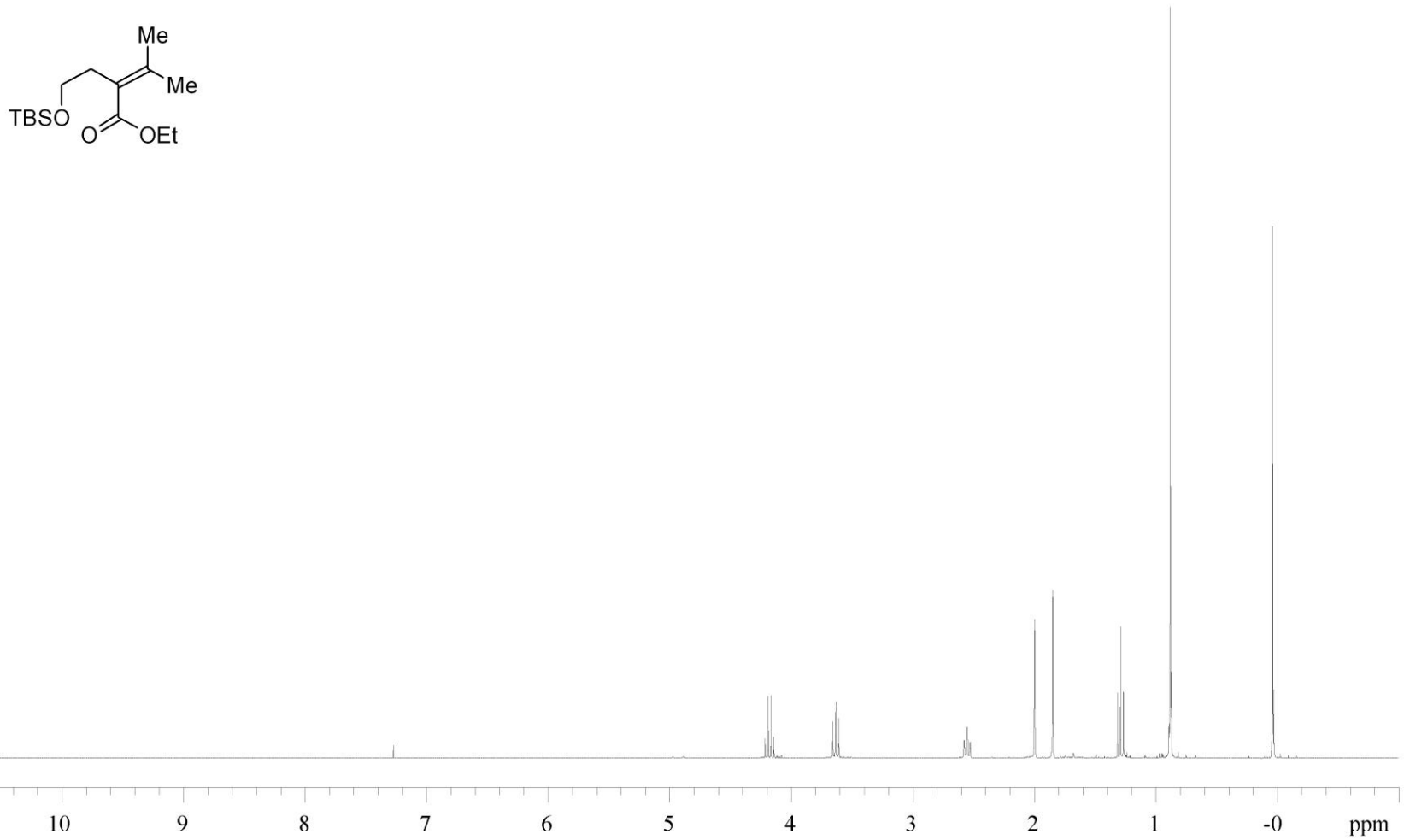

PMBO
H
O
BPSO
O
StBu
ppm
200
180
160
140
120
100
80
60
40
20

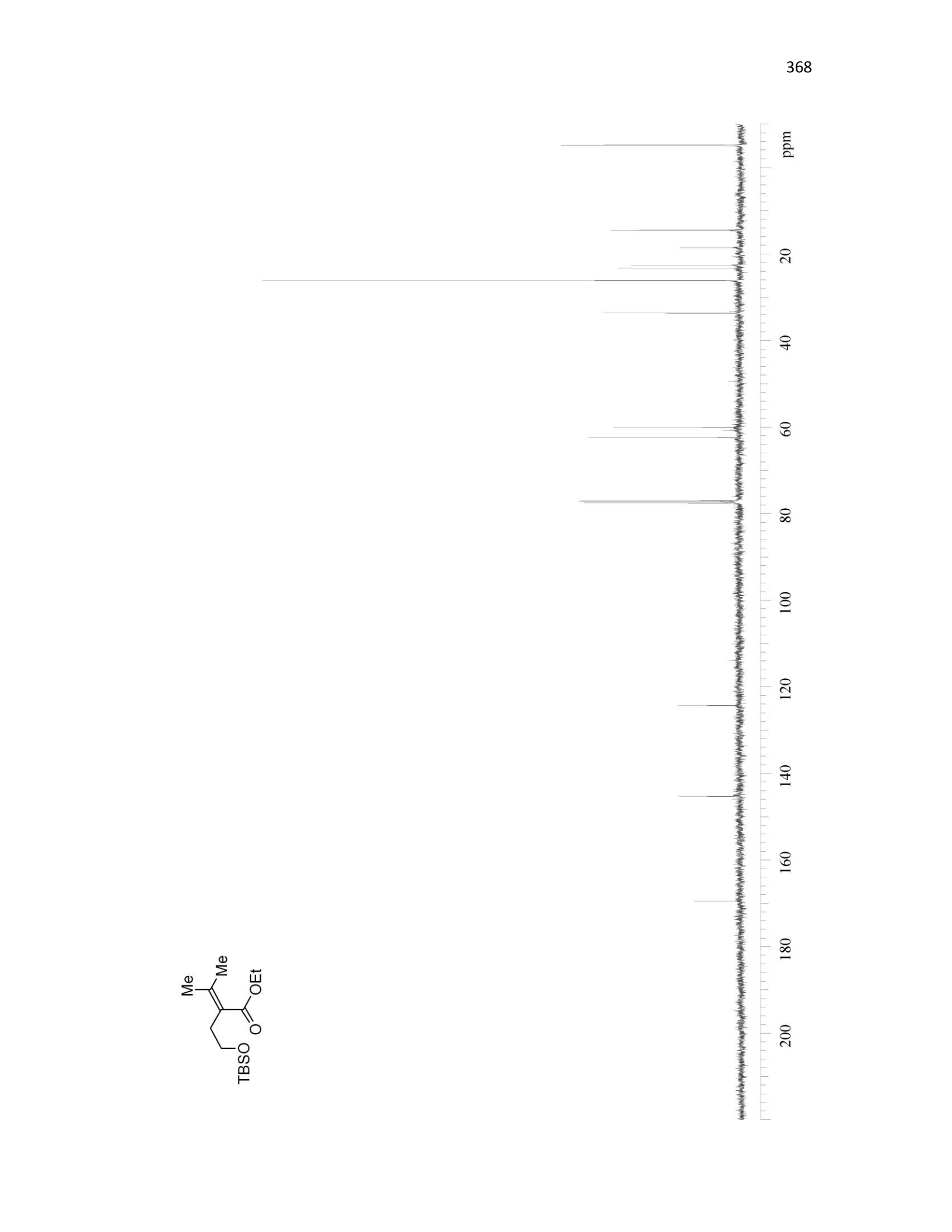

Me
OEt
O
TBSO
10
9
8
7
6
5
4
3
2
1
-0
ppm

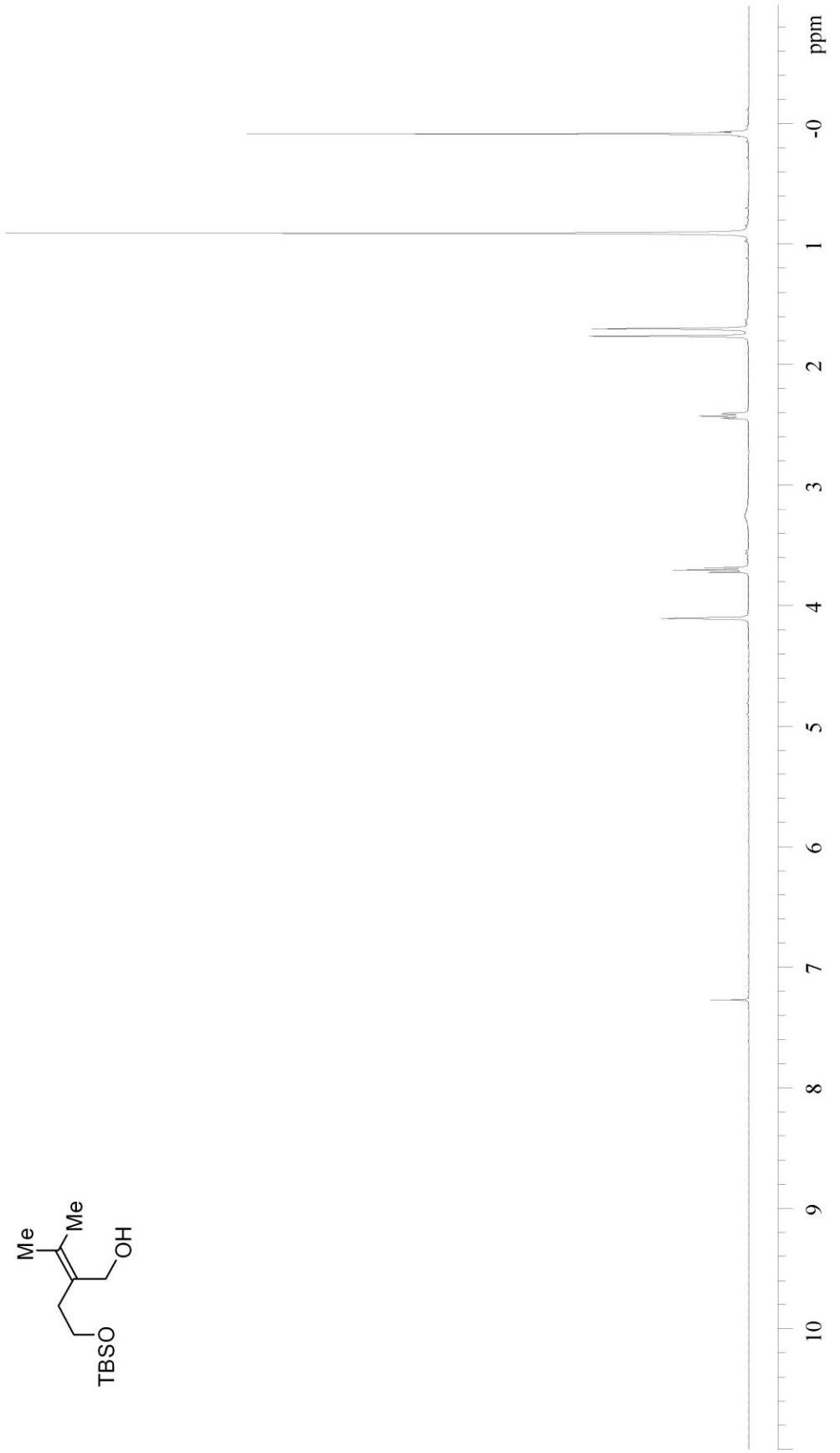

Me
OEt
O
TBSO
ppm
200
180
160
140
120
100
80
60
40
20

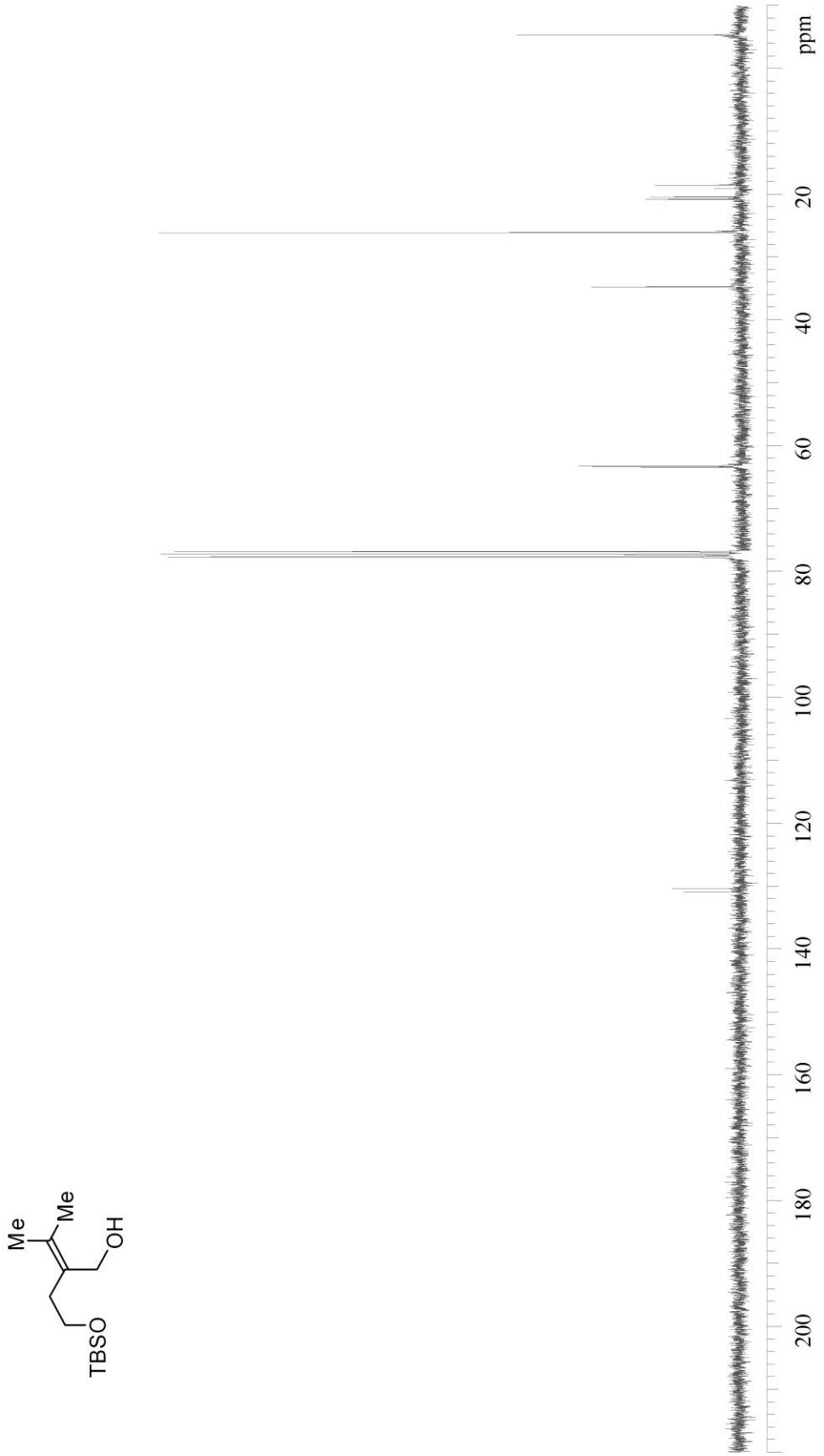

TBSO
I
10
9
8
7
6
5
4
3
2
1
-0
ppm

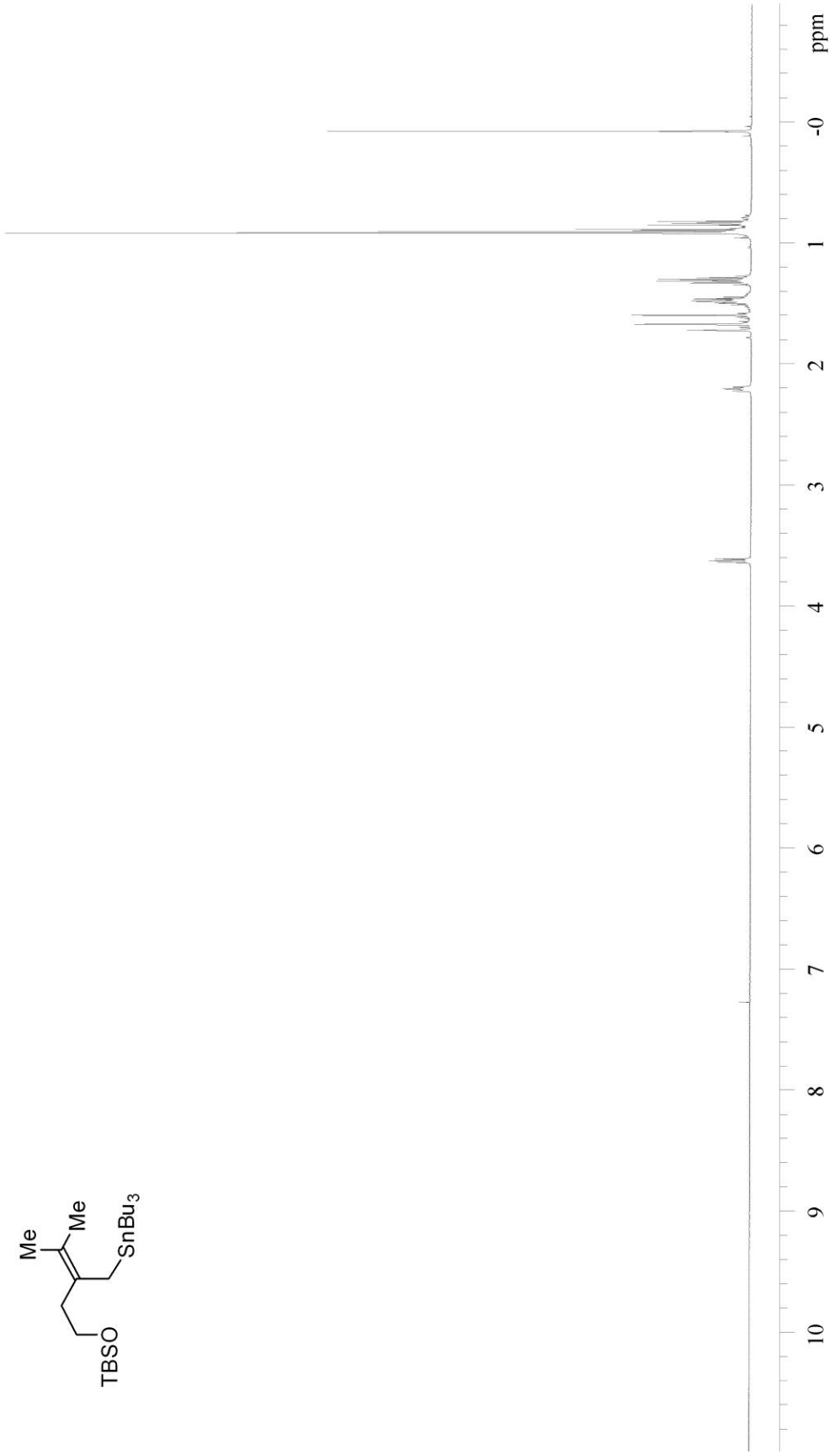

TBSO
I
200
180
160
140
120
100
80
60
40
20
ppm

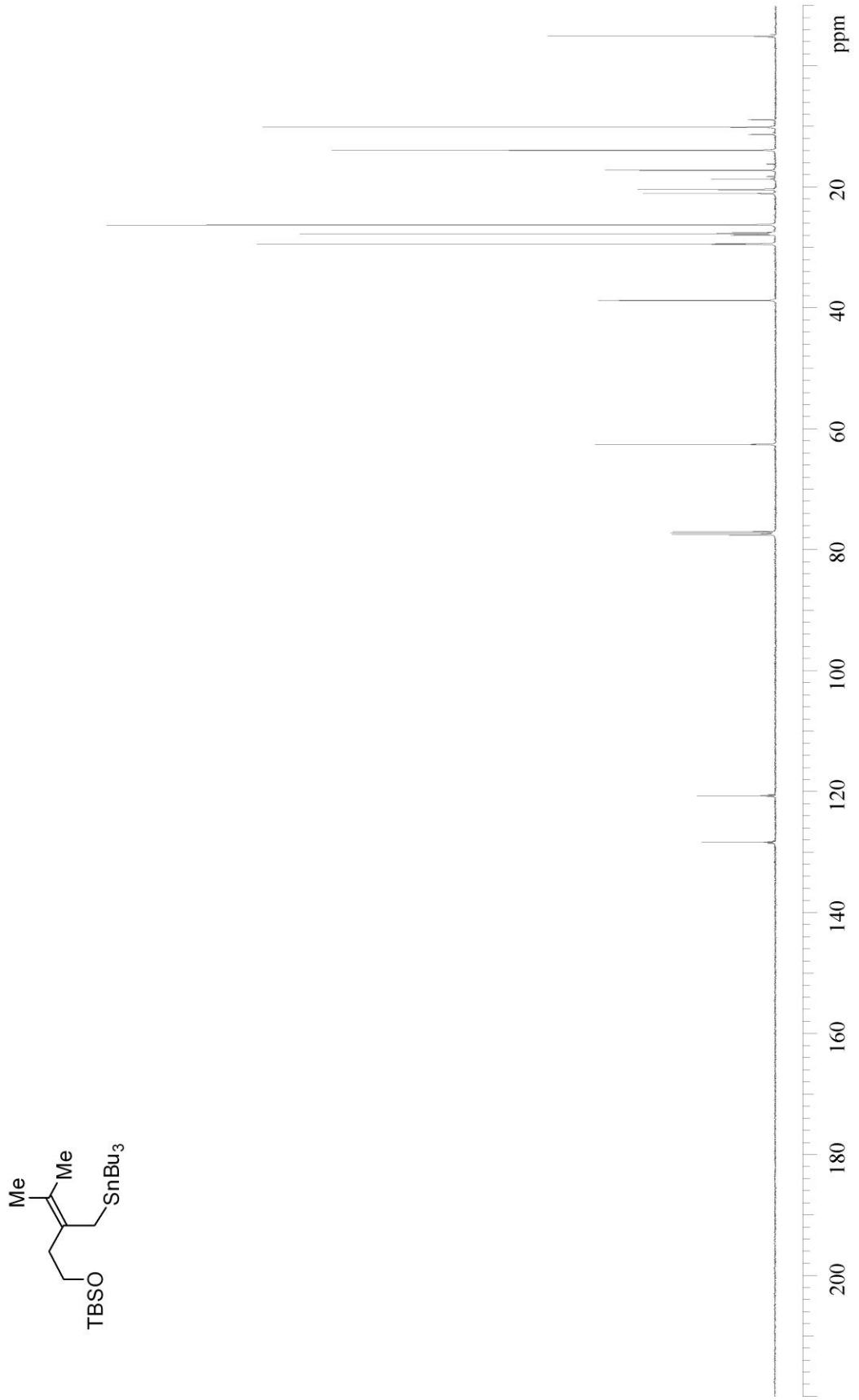

Me
Me
TBSO
O
OEt
10
9
8
7
6
5
4
3
2
1
-0
ppm

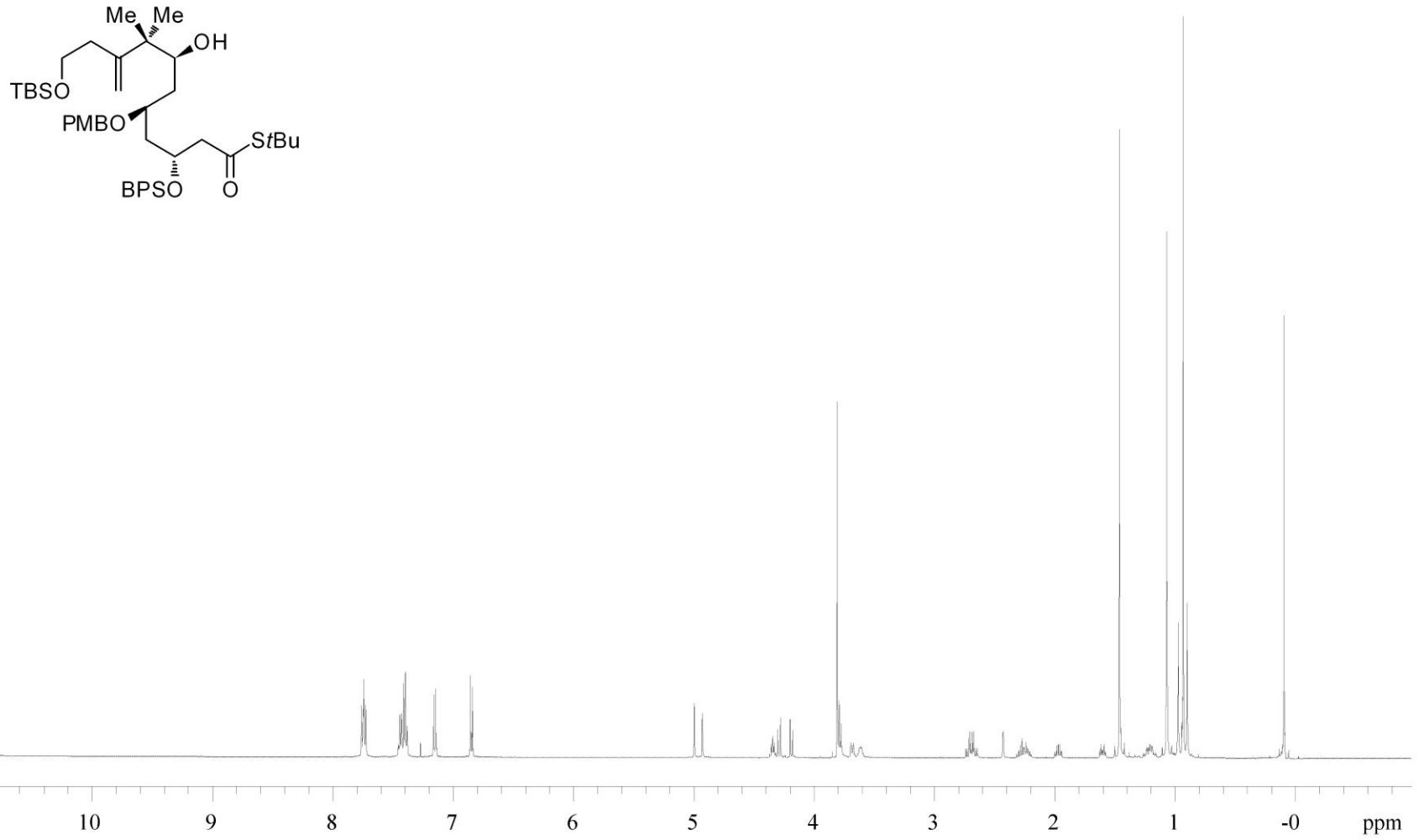

Me
Me
OEt
O
TBSO
ppm
200
180
160
140
120
100
80
60
40
20

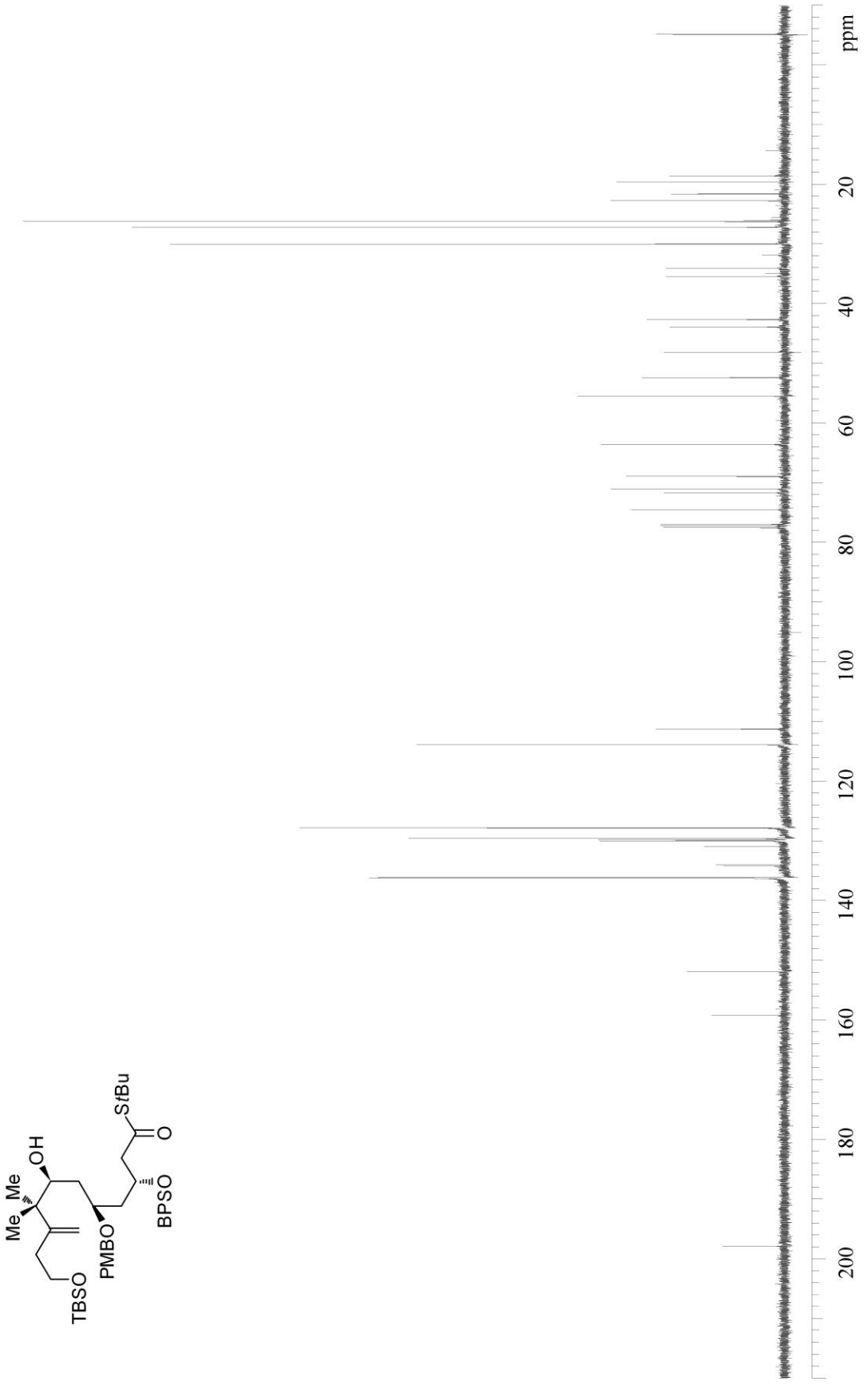

Me
Me
OH
TBSO
10
9
8
7
6
5
4
3
2
1
-0
ppm

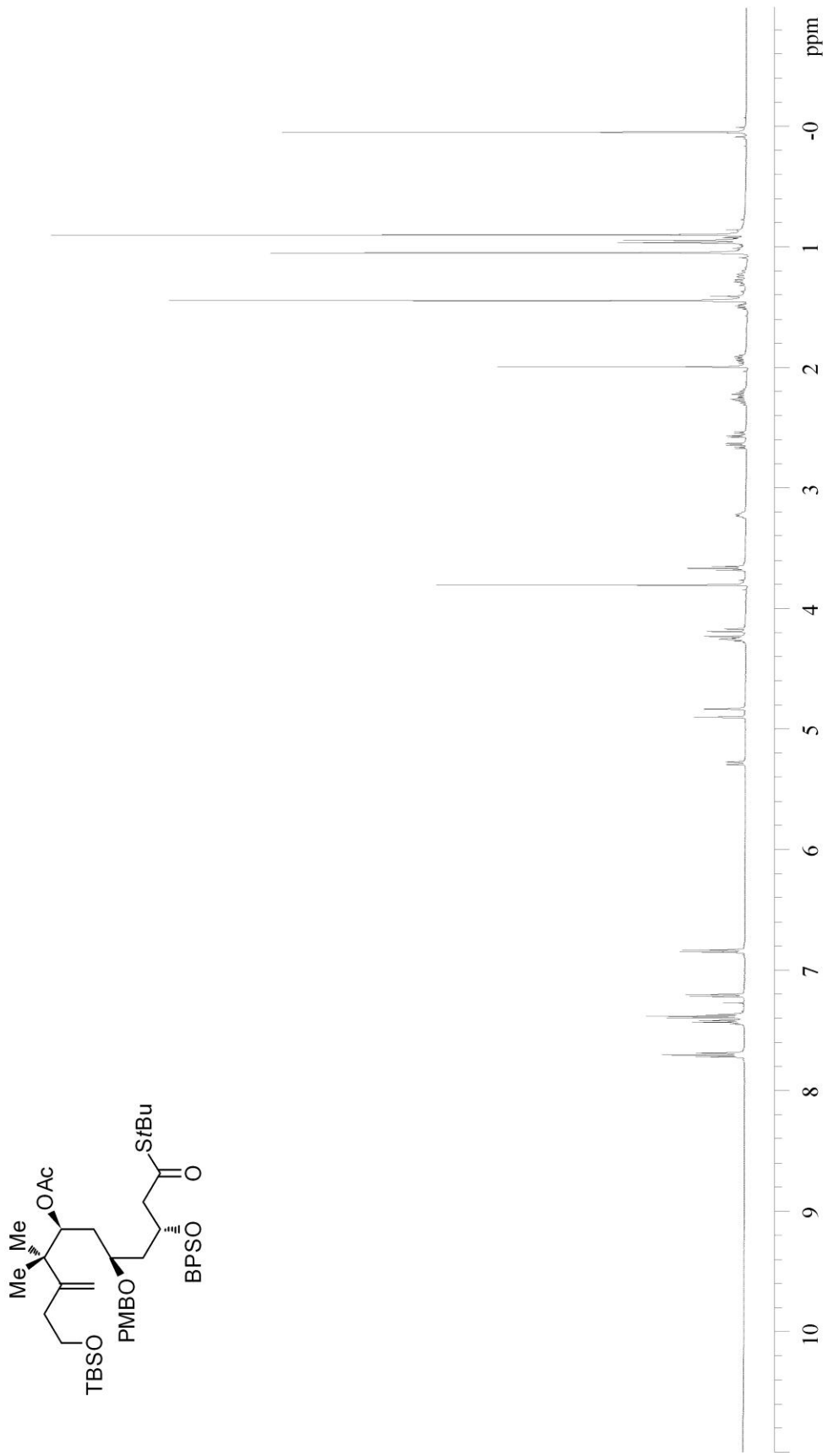

Me
Me
OH
TBSO
ppm
200
180
160
140
120
100
80
60
40
20

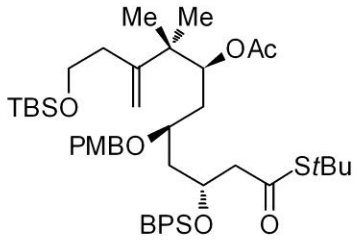

Me
Me
SnBu3
TBSO
10
9
8
7
6
5
4
3
2
1
-0
ppm

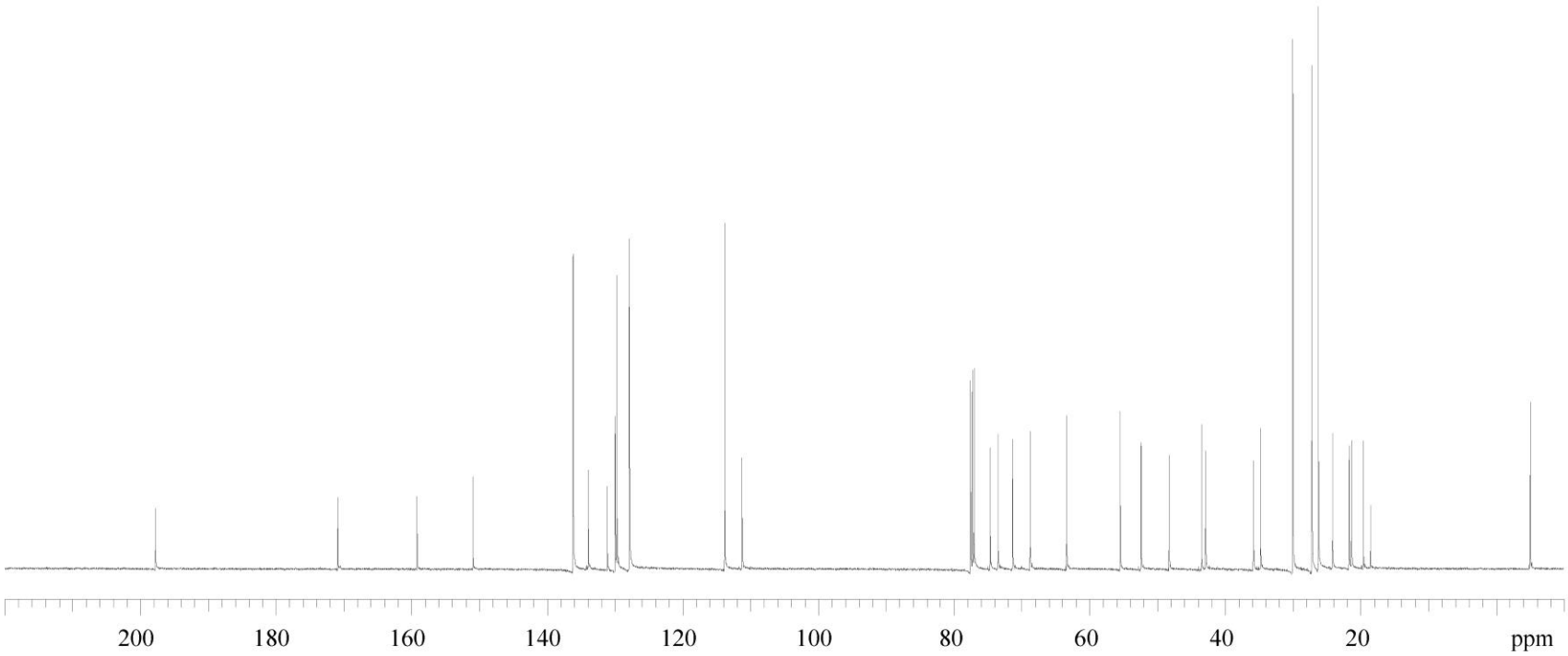

Me
Me
SnBu3
TBSO
ppm
200
180
160
140
120
100
80
60
40
20

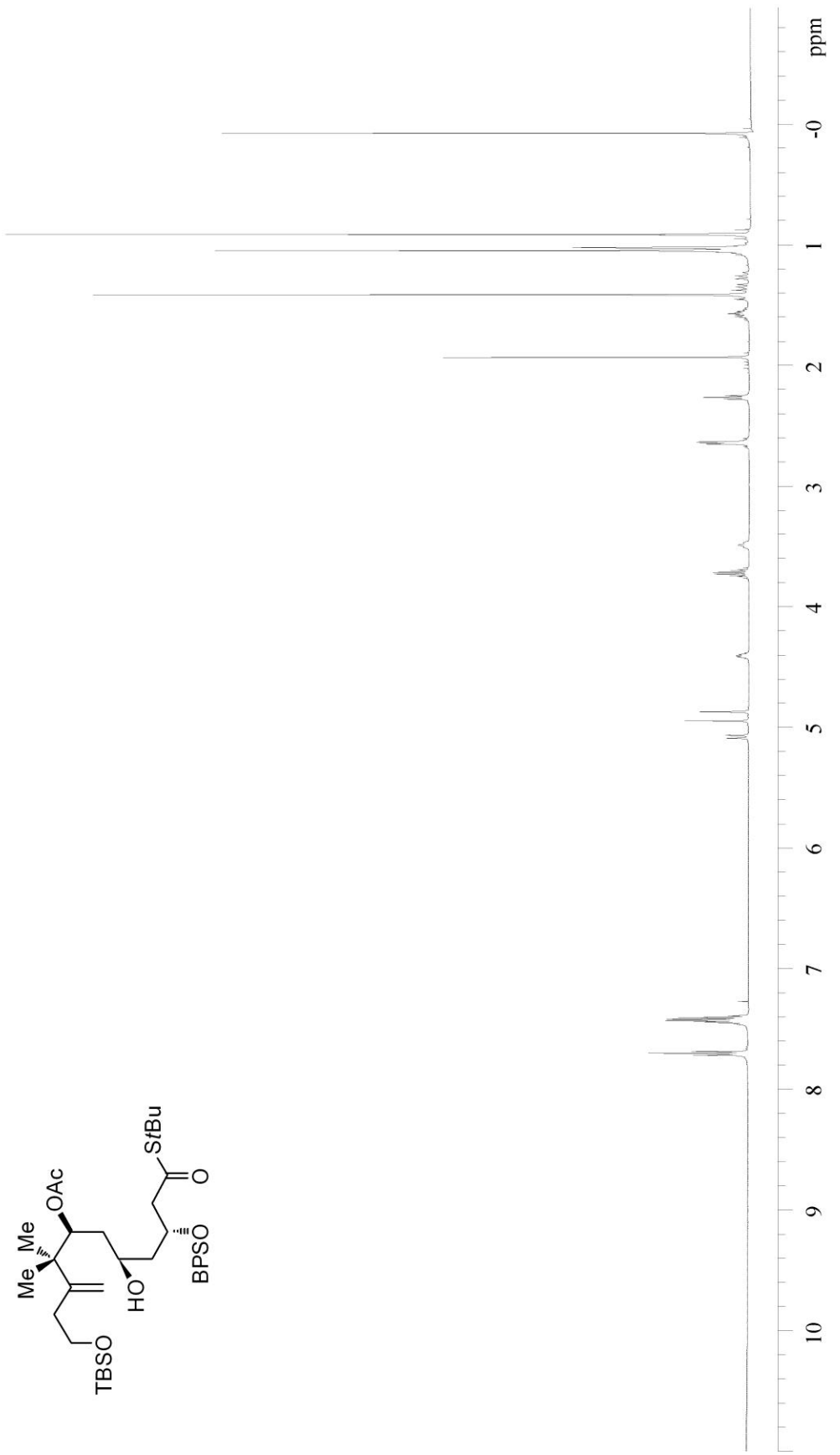

Me
Me
OH
TBSO
PMBO
S*t*Bu
BPSO
O
10
9
8
7
6
5
4
3
2
1
-0
ppm

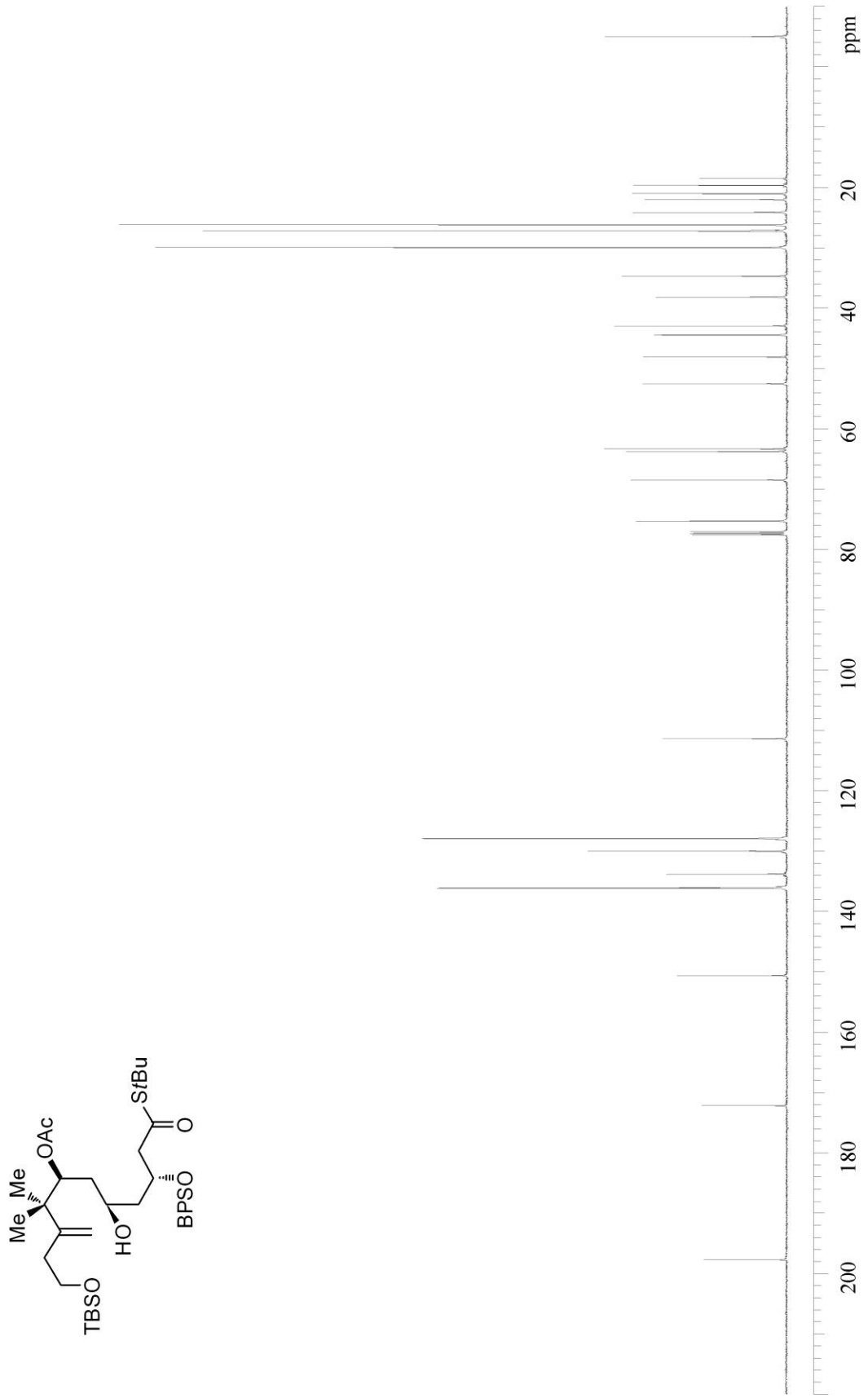

ppm
20
40
60
80
100
120
140
160
180
200
TBSO
PMBO
BPSO
OH
Me
Me
StBu
O

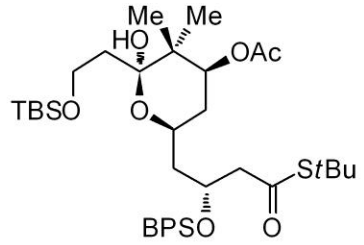

TBSO
Me
Me
OAc
PMBO
BPSO
O
StBu
10
9
8
7
6
5
4
3
2
1
-0
ppm


Me
Me
OAc
TBSO
PMBO
S*t*Bu
BPSO
O

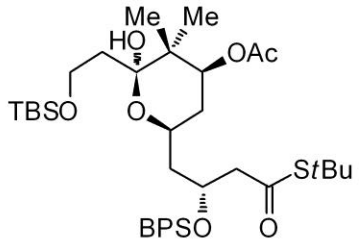

200
180
160
140
120
100
80
60
40
20
ppm

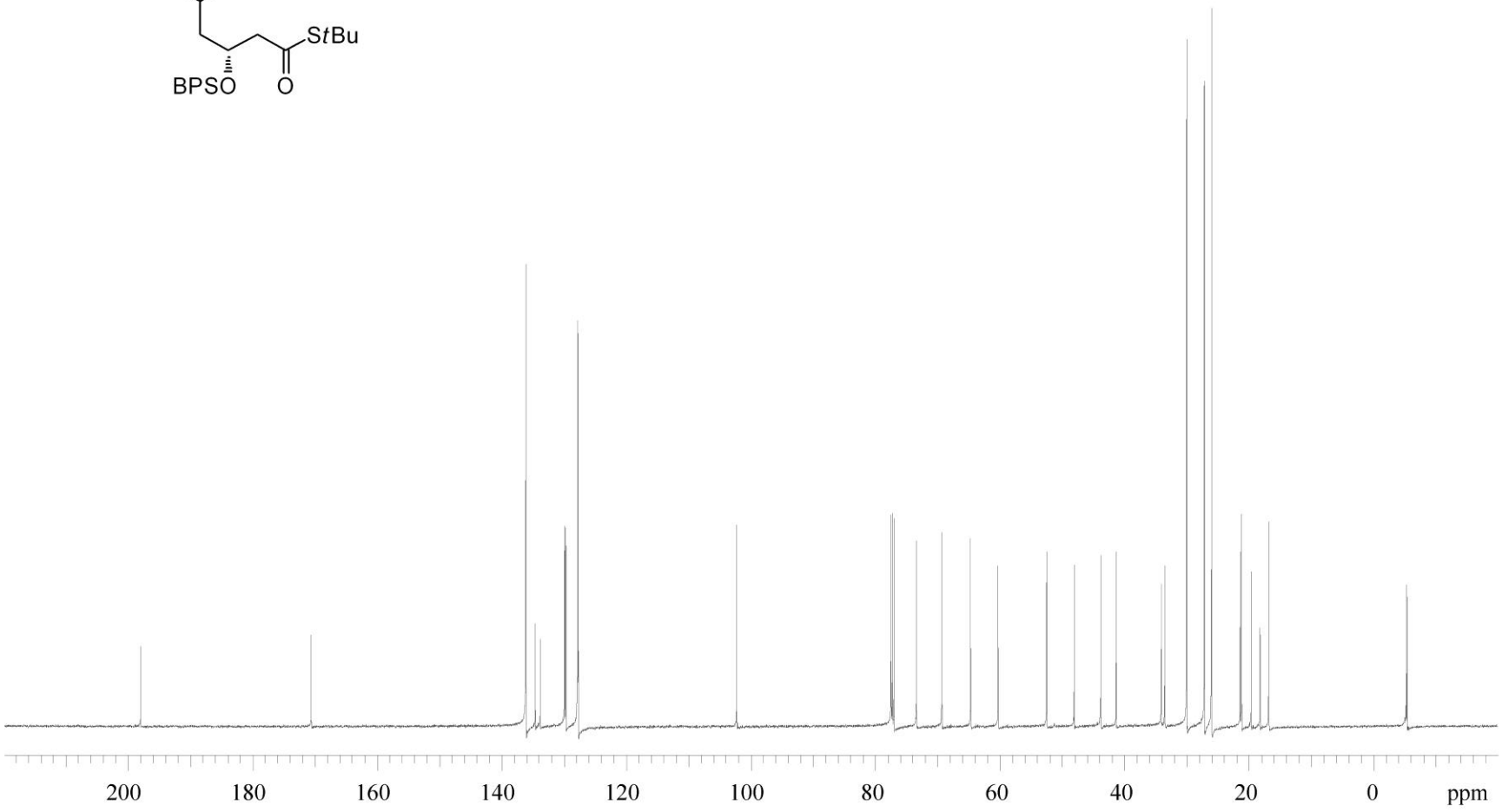

TBSO
Me
Me
OAc
HO
BPSO
O
StBu
10
9
8
7
6
5
4
3
2
1
-0
ppm

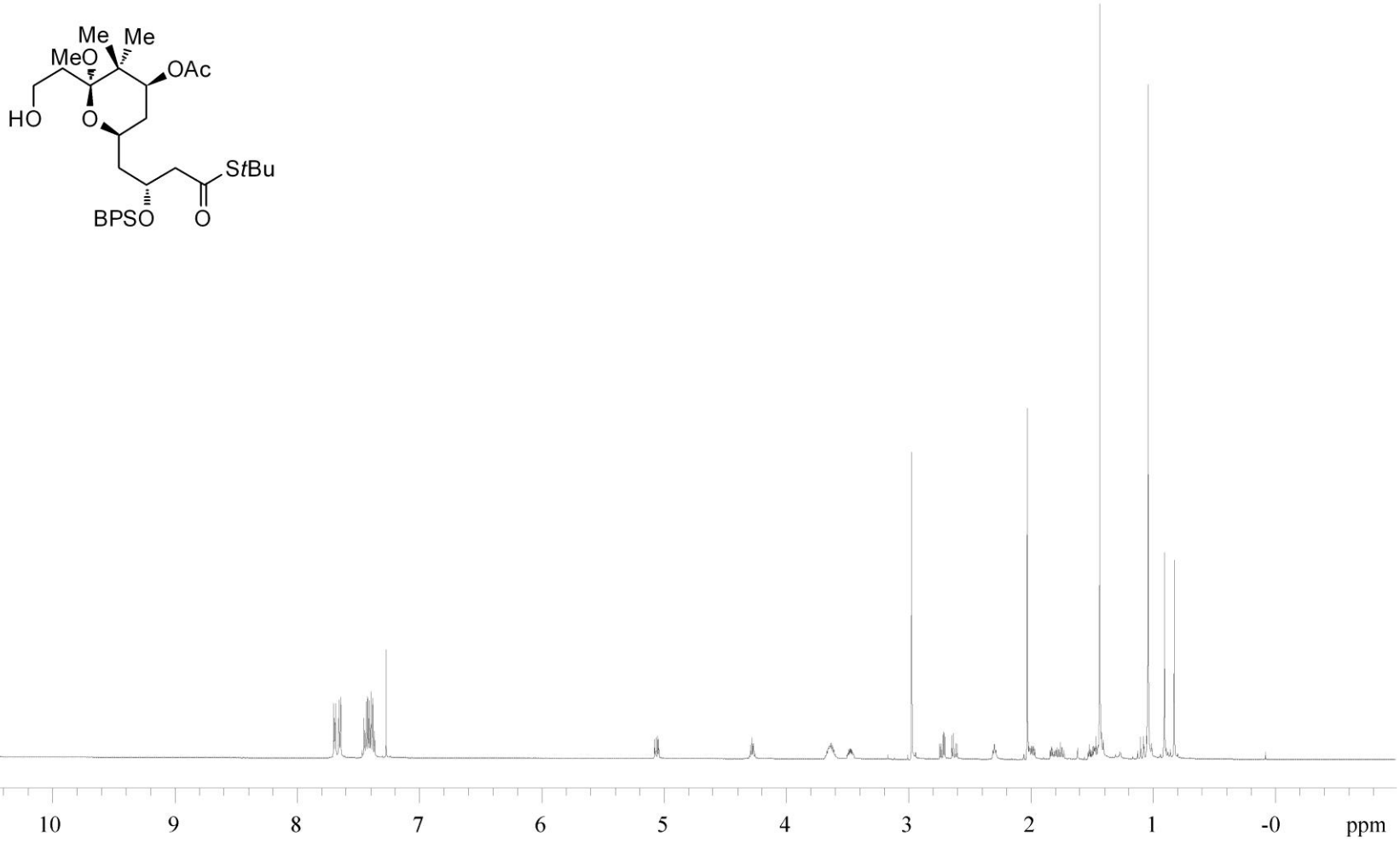

TBSO
Me
Me
OAc
HO
BPSO
StBu
O
ppm
200
180
160
140
120
100
80
60
40
20

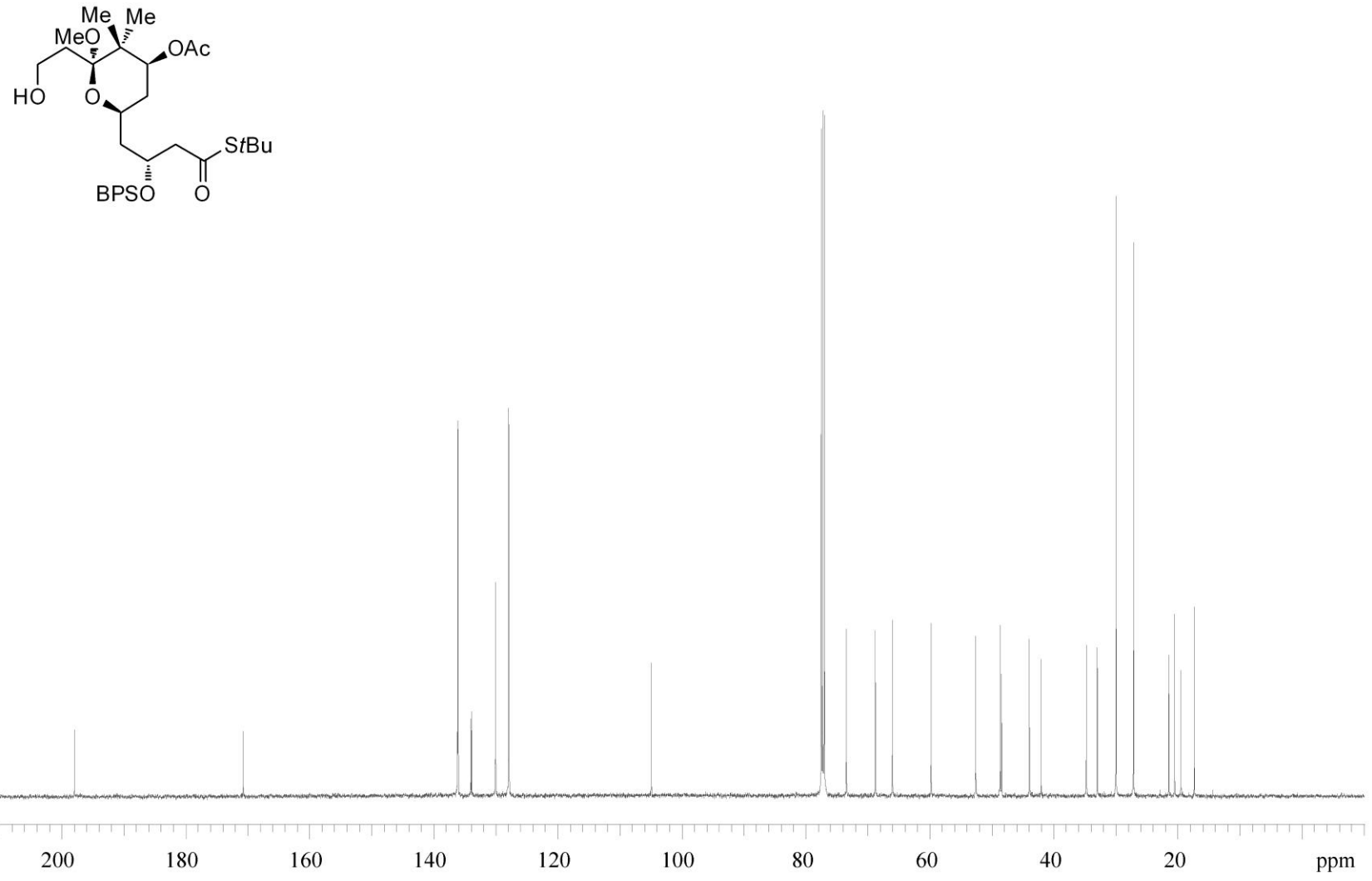

Me
Me
HO
OAc
TBSO
O
StBu
BPSO
O

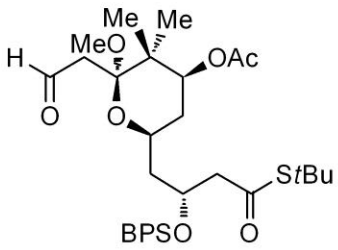
10
9
8
7
6
5
4
3
2
1
-0
ppm


Me
Me
HO
OAc
TBSO
O
StBu
BPSO
O

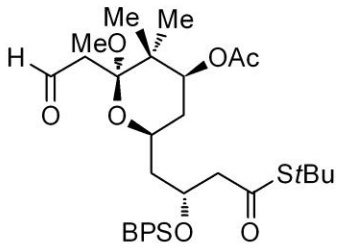

200
180
160
140
120
100
80
60
40
20
0
ppm

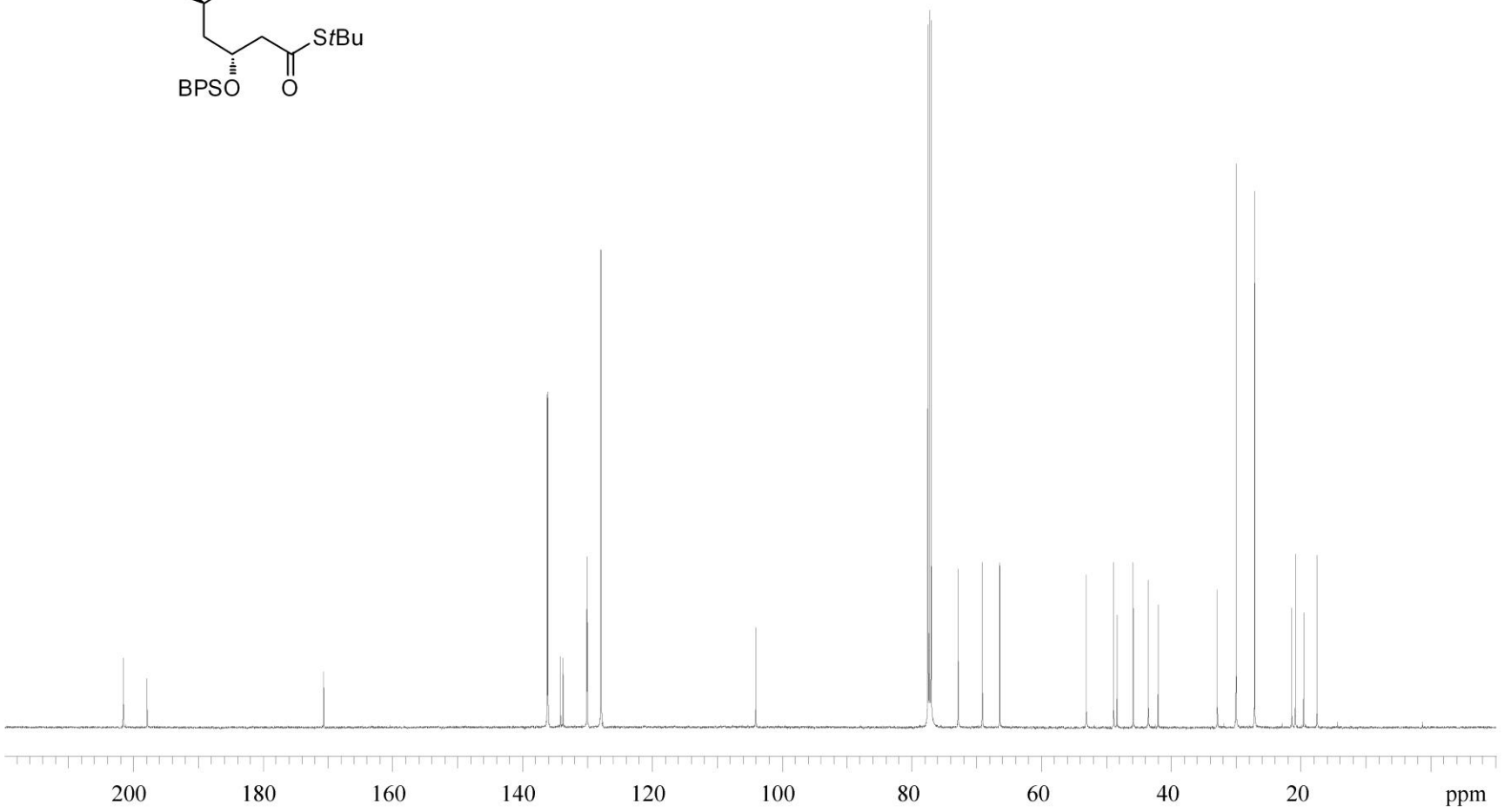

Me
Me
MeO
OAc
HO
O
StBu
BPSO
O
10
9
8
7
6
5
4
3
2
1
-0
ppm

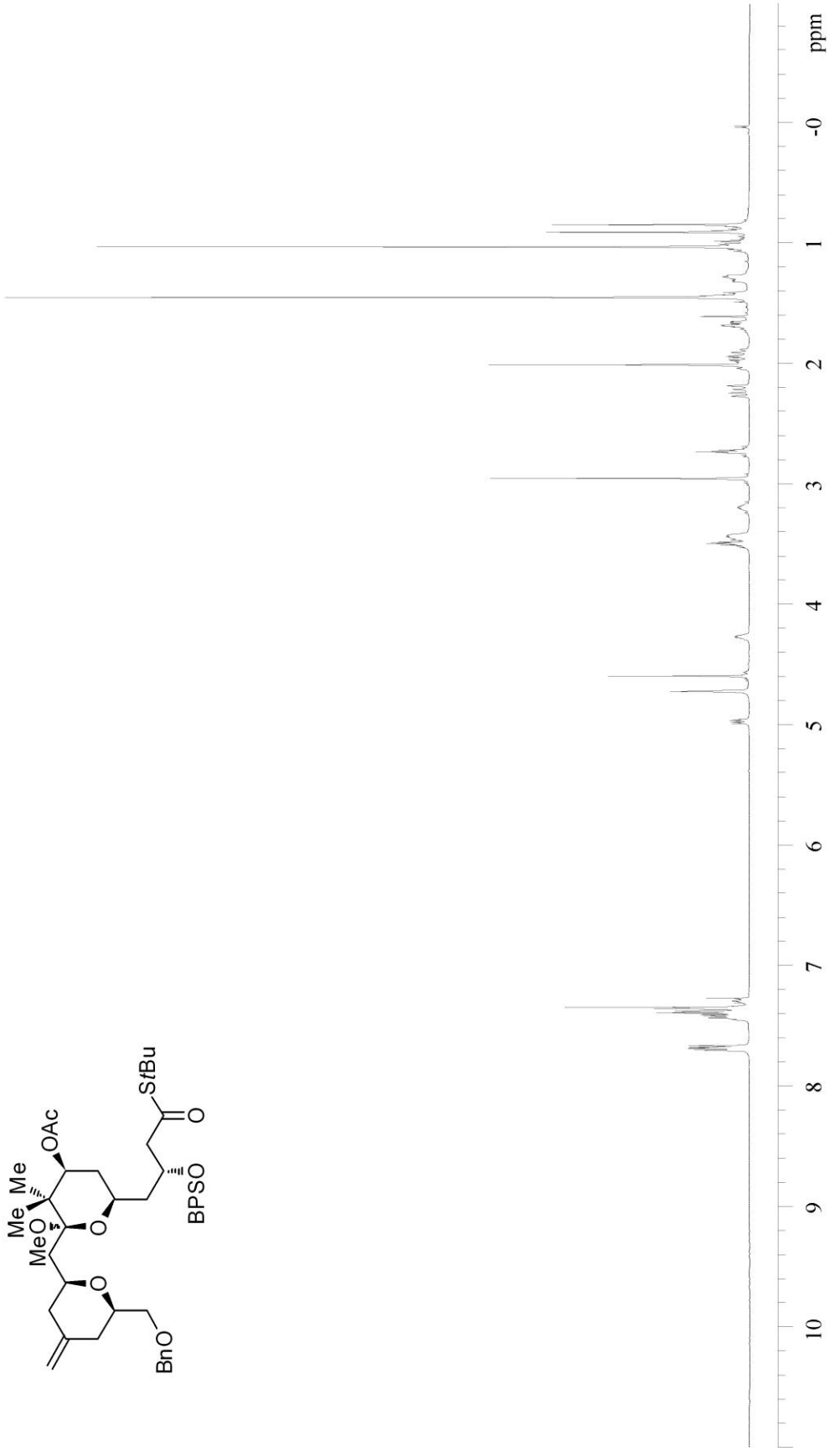

Me
Me
MeO
OAc
HO
O
StBu
BPSO
O
200
180
160
140
120
100
80
60
40
20
ppm

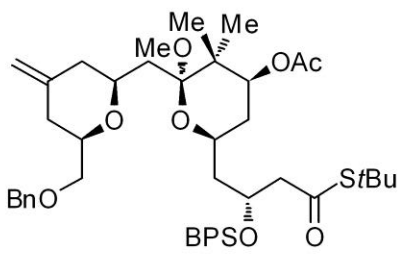

Me
Me
MeO
H
OAc
O
O
StBu
BPSO
O

10
9
8
7
6
5
4
3
2
1
-0
ppm

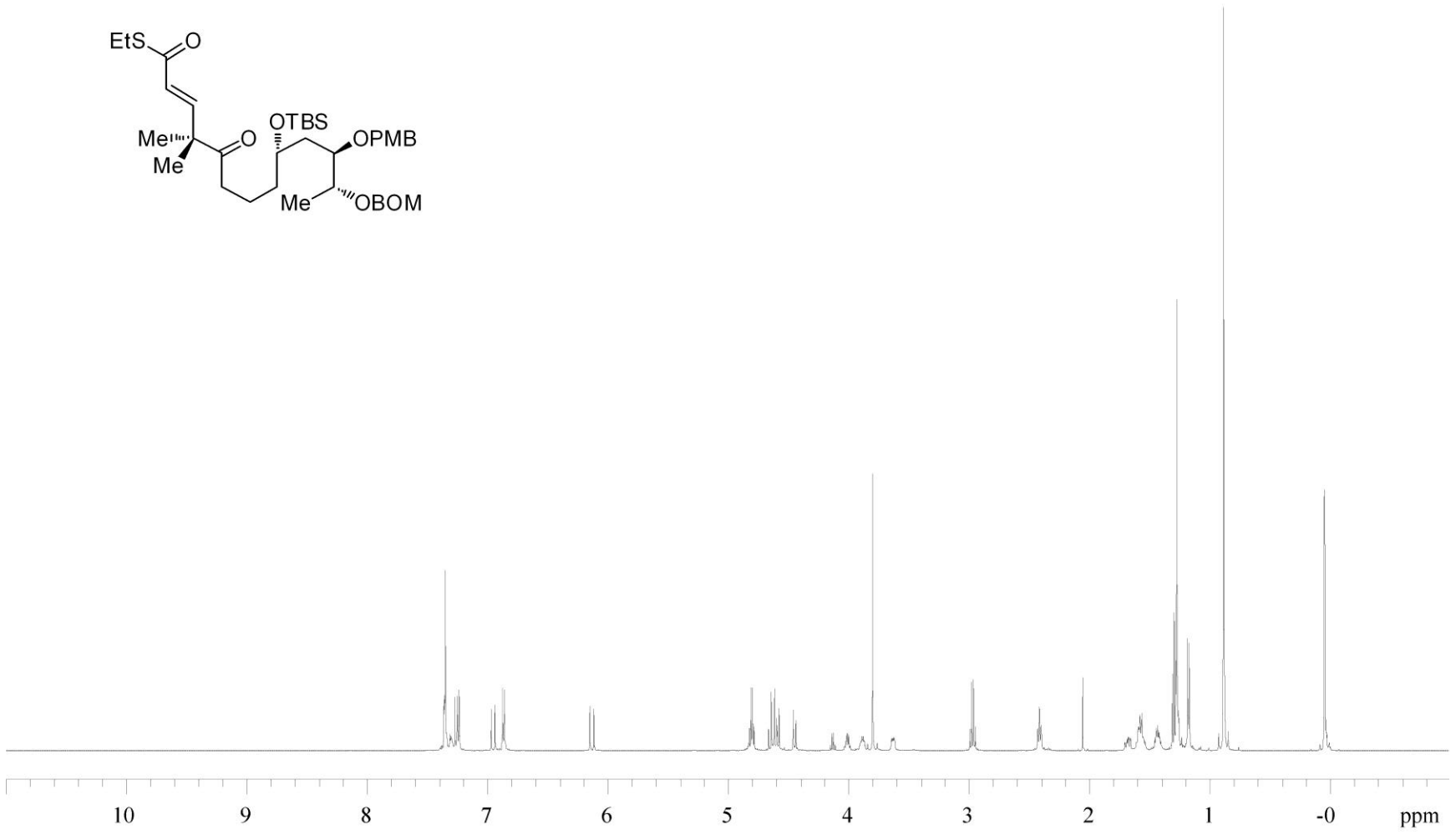

Me
Me
MeO
H
OAc
O
O
S*t*Bu
BPSO
O

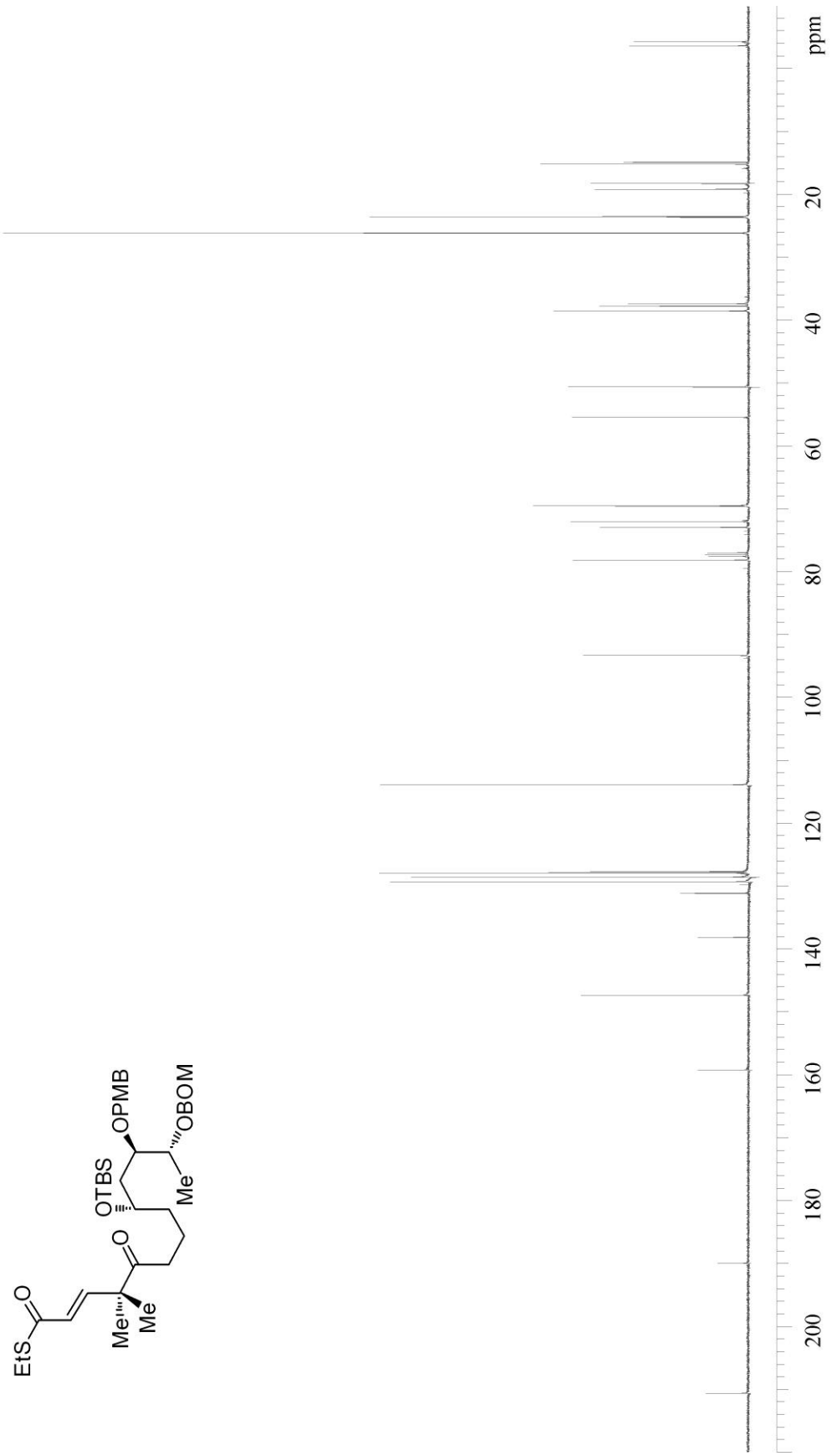

200
180
160
140
120
100
80
60
40
20
ppm

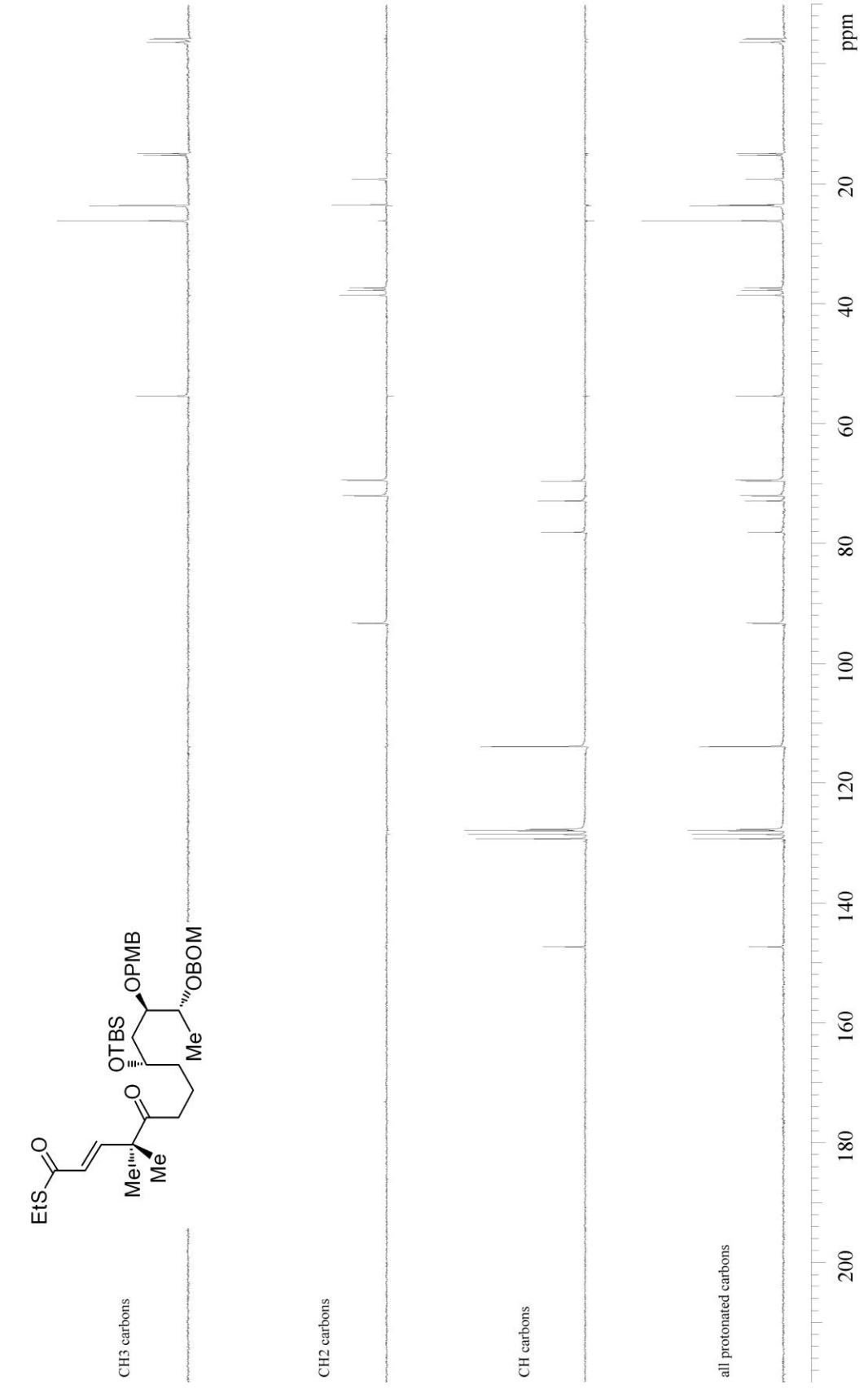

Me
Me
MeO
OAc
O
O
BnO
BPSO
O
StBu
10
9
8
7
6
5
4
3
2
1
-0
ppm

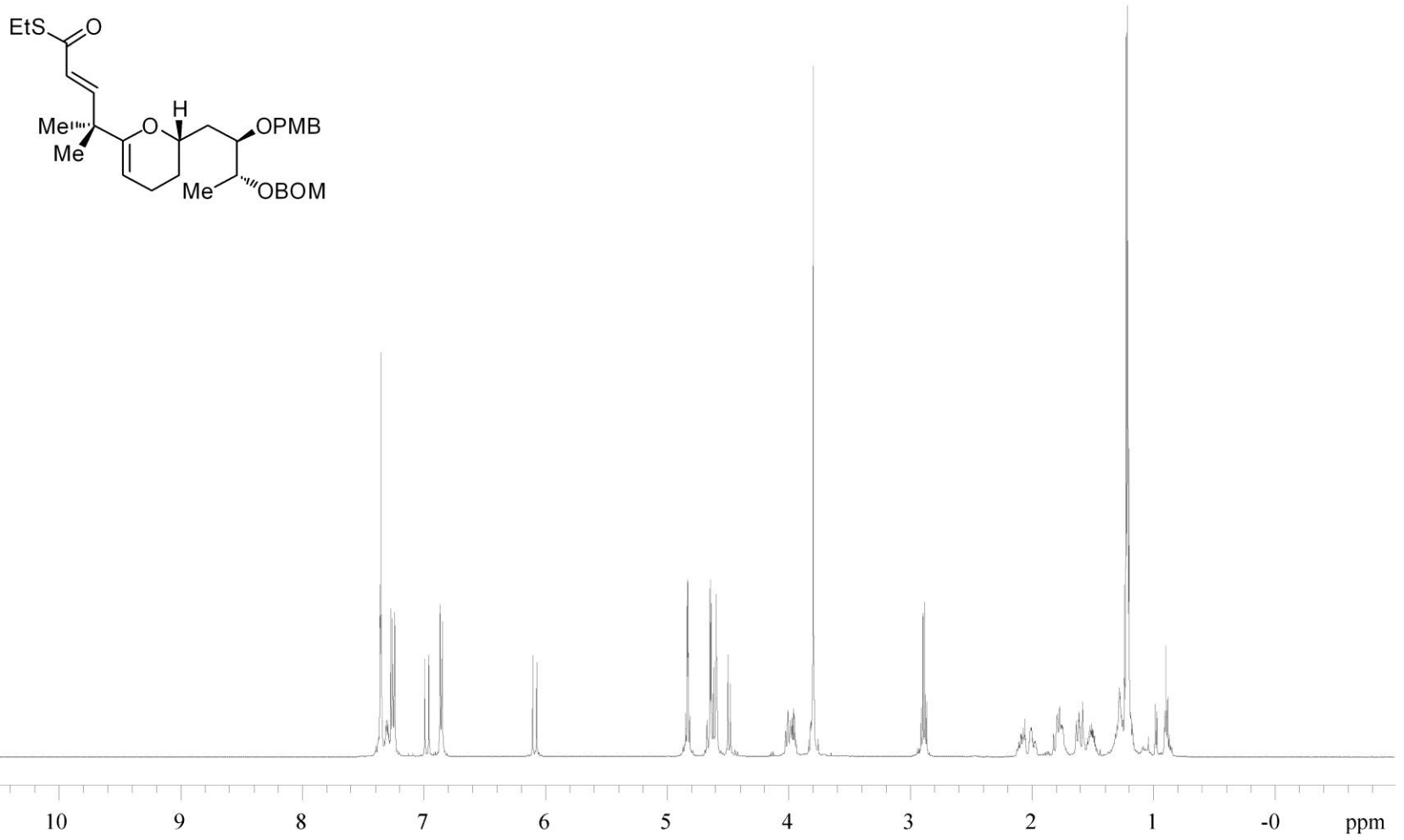

Me
Me
MeO
OAc
O
O
BnO
StBu
BPSO
O

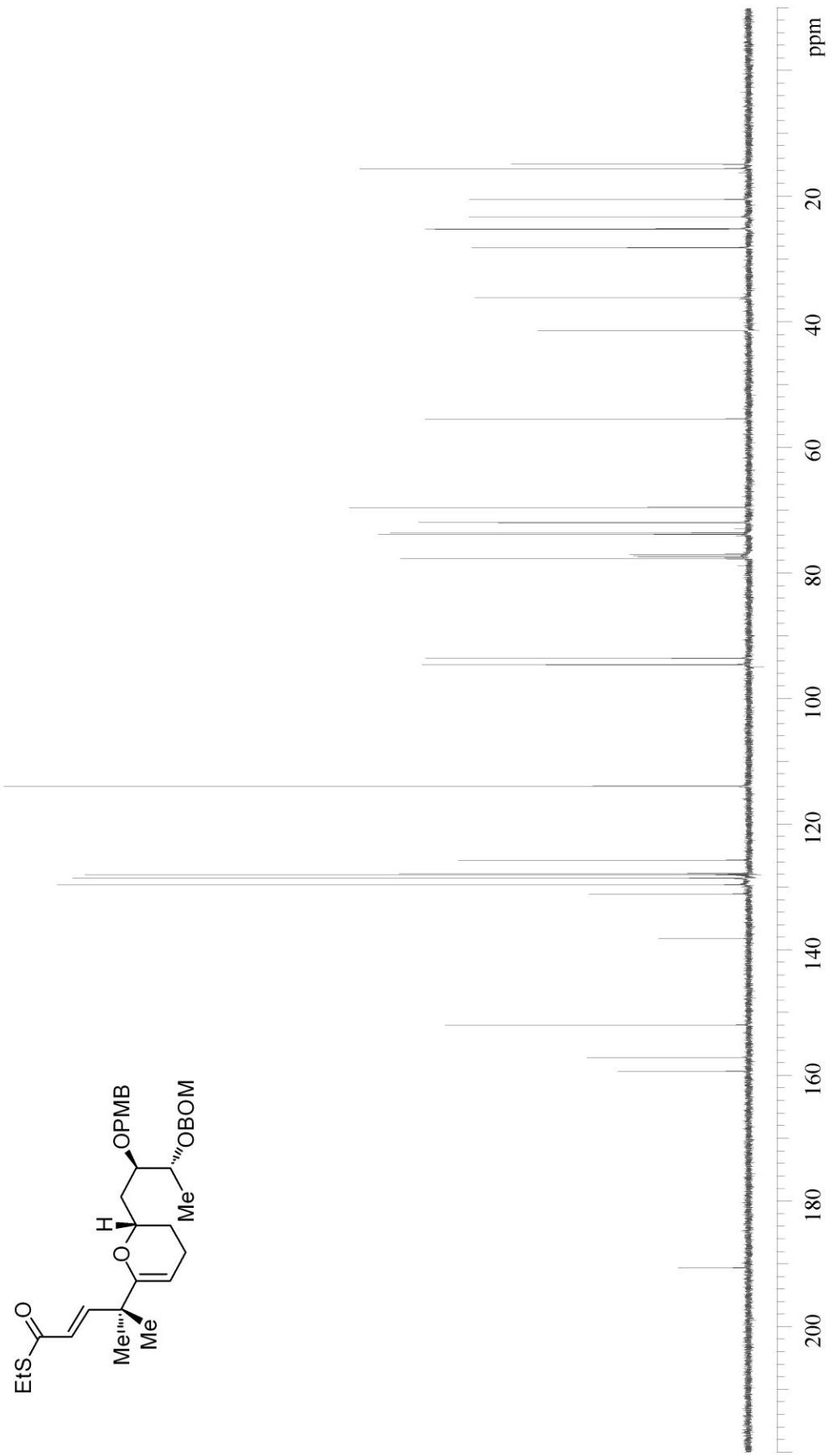
200
180
160
140
120
100
80
60
40
20
ppm

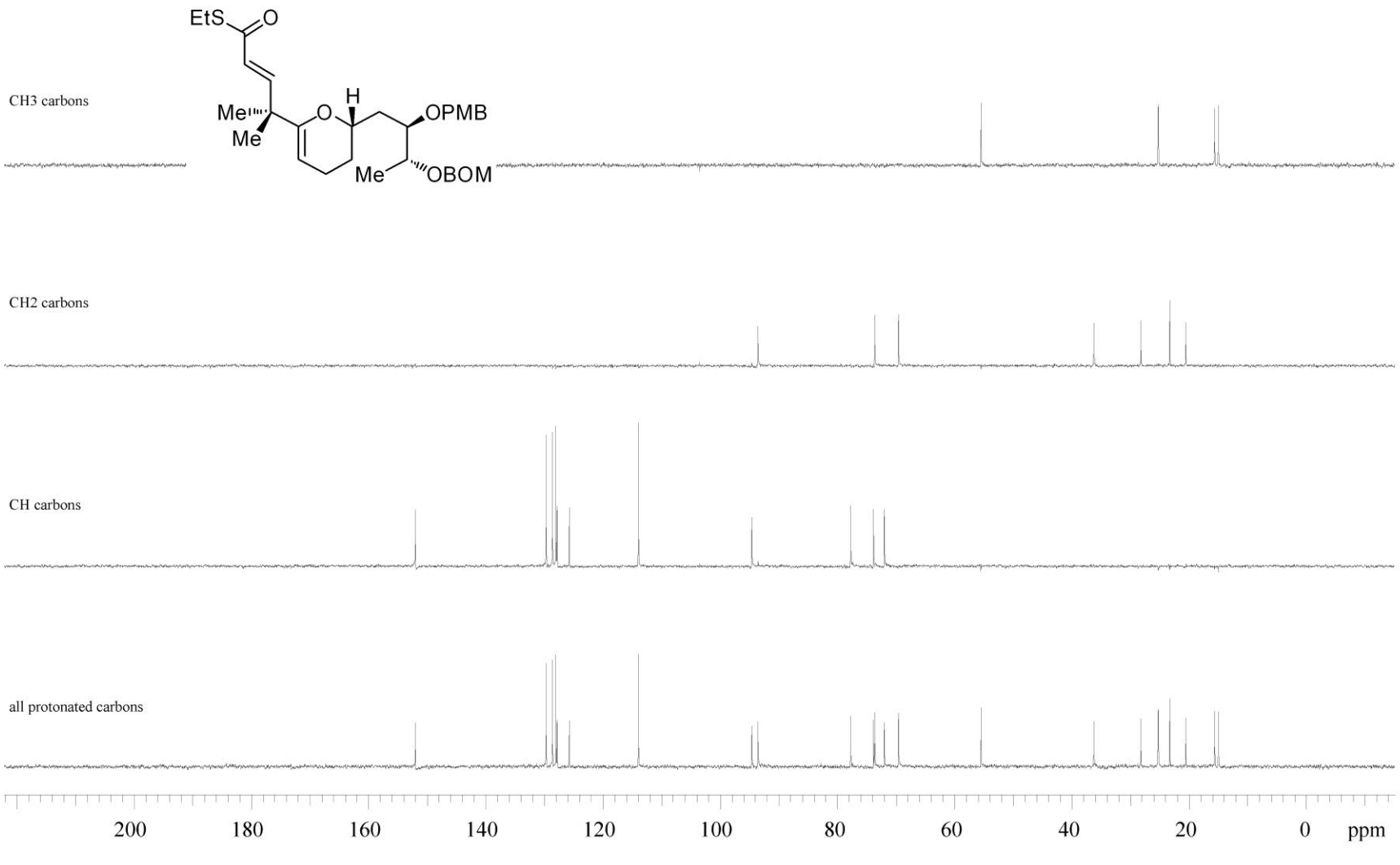

EtS
O
Me
Me
O
OTBS
OPMB
Me
OBOM
10
9
8
7
6
5
4
3
2
1
-0
ppm

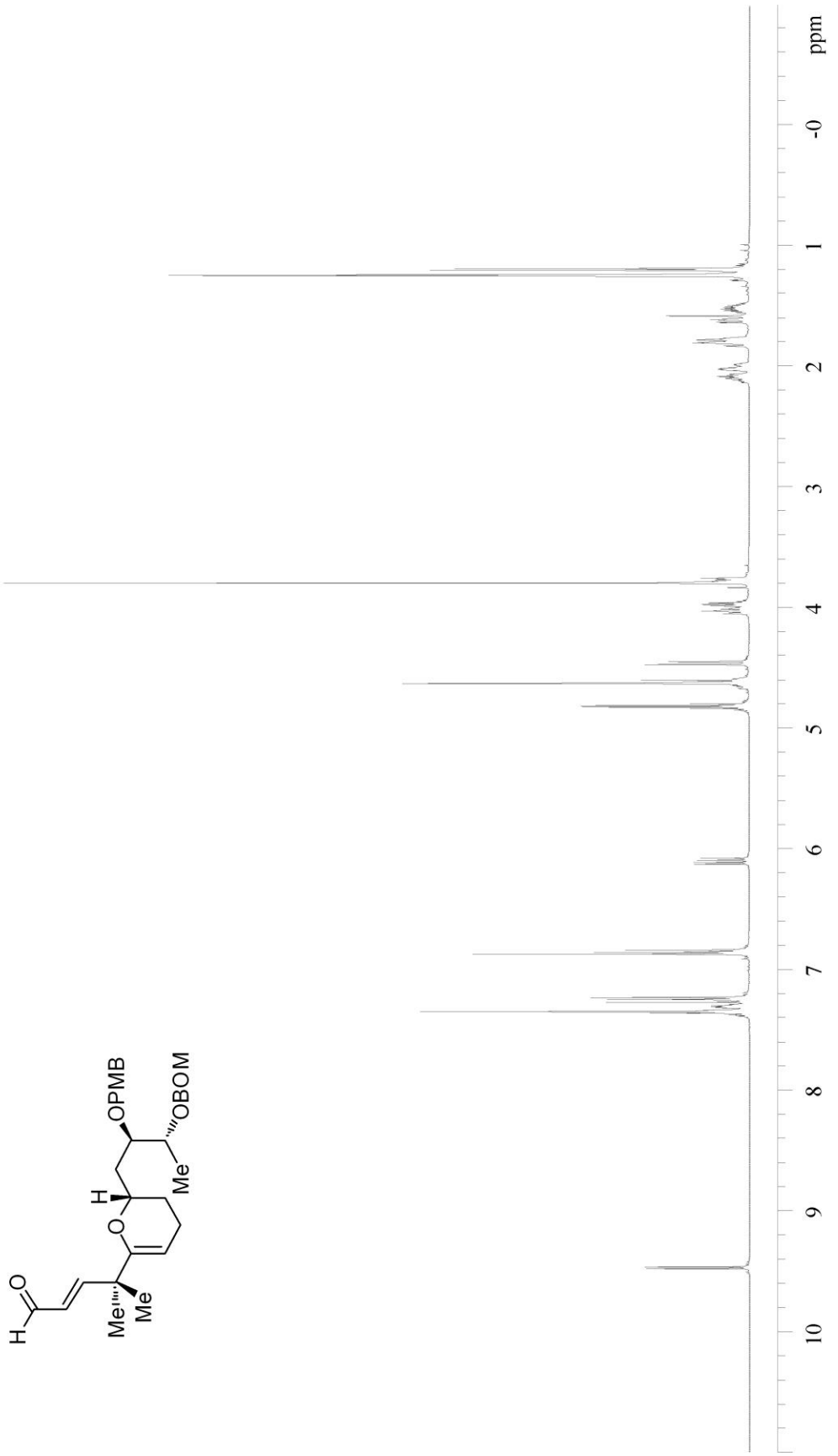

EtS
O
Me
Me
O
OTBS
OPMB
Me
OBOM
200
180
160
140
120
100
80
60
40
20
ppm

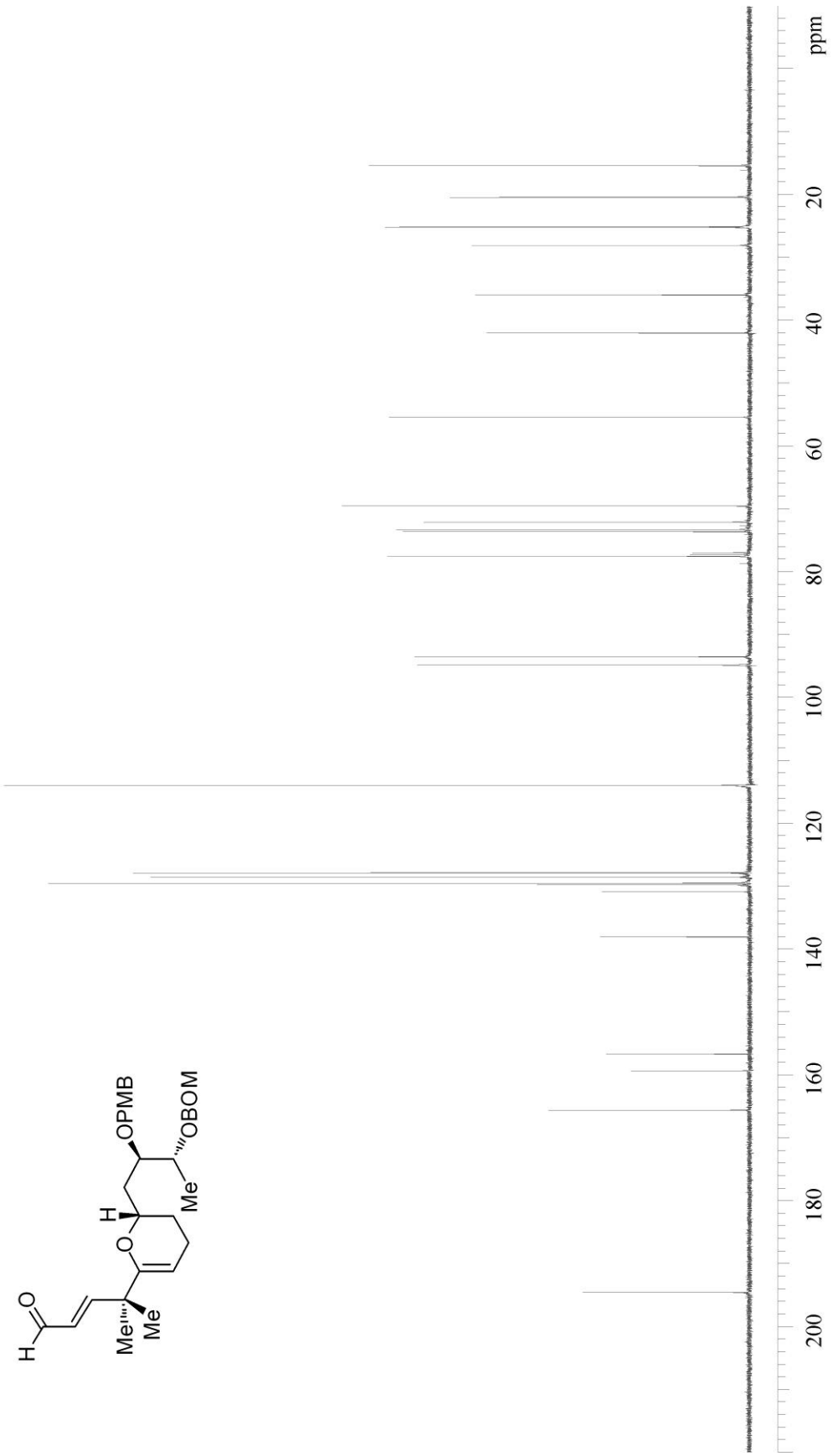

EtS
O
Me
Me
O
OTBS
OPMB
Me
OBOM
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
ppm

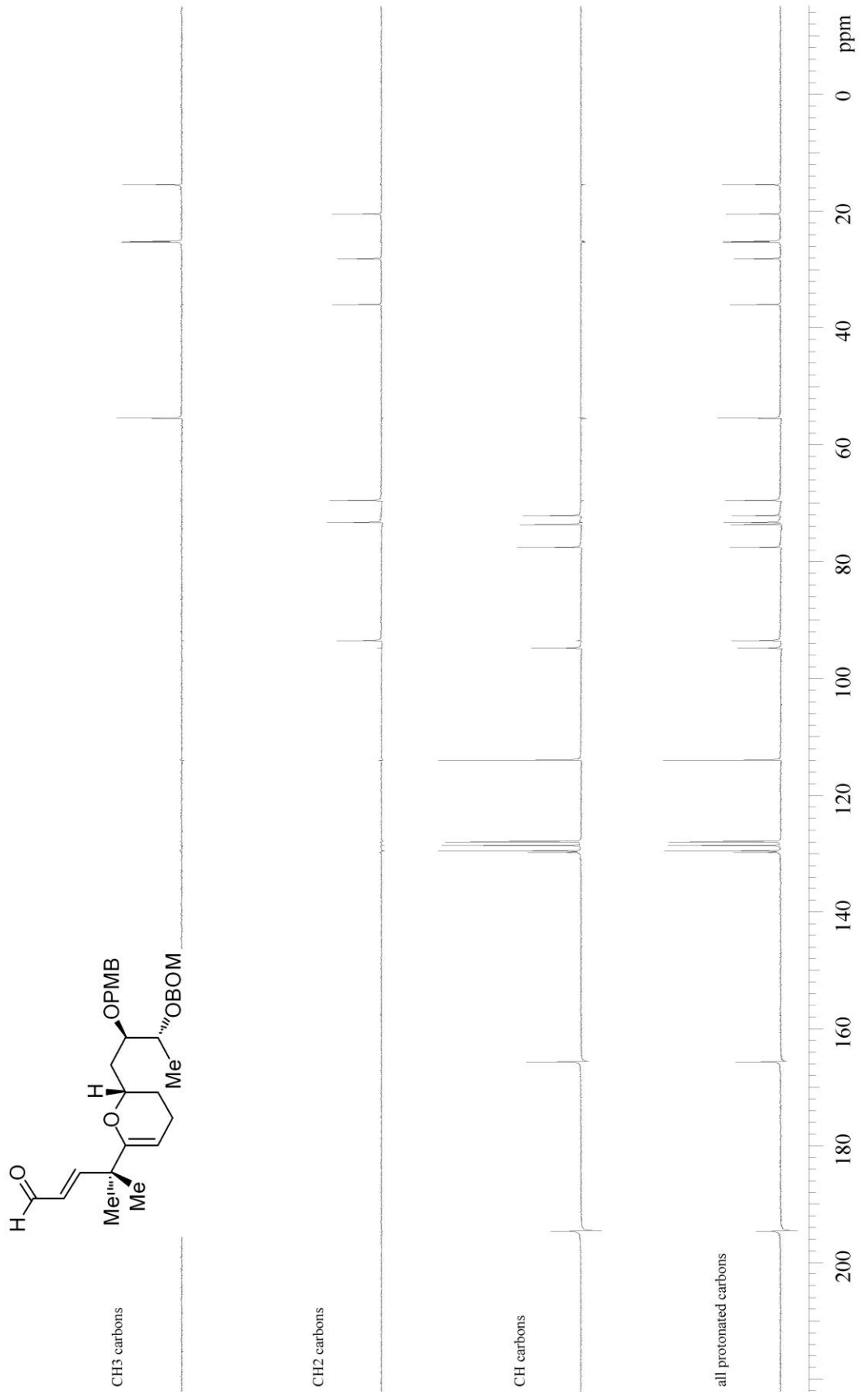

EtS
O
H
O
Me
Me
OPMB
Me
OBOM
10
9
8
7
6
5
4
3
2
1
-0
ppm

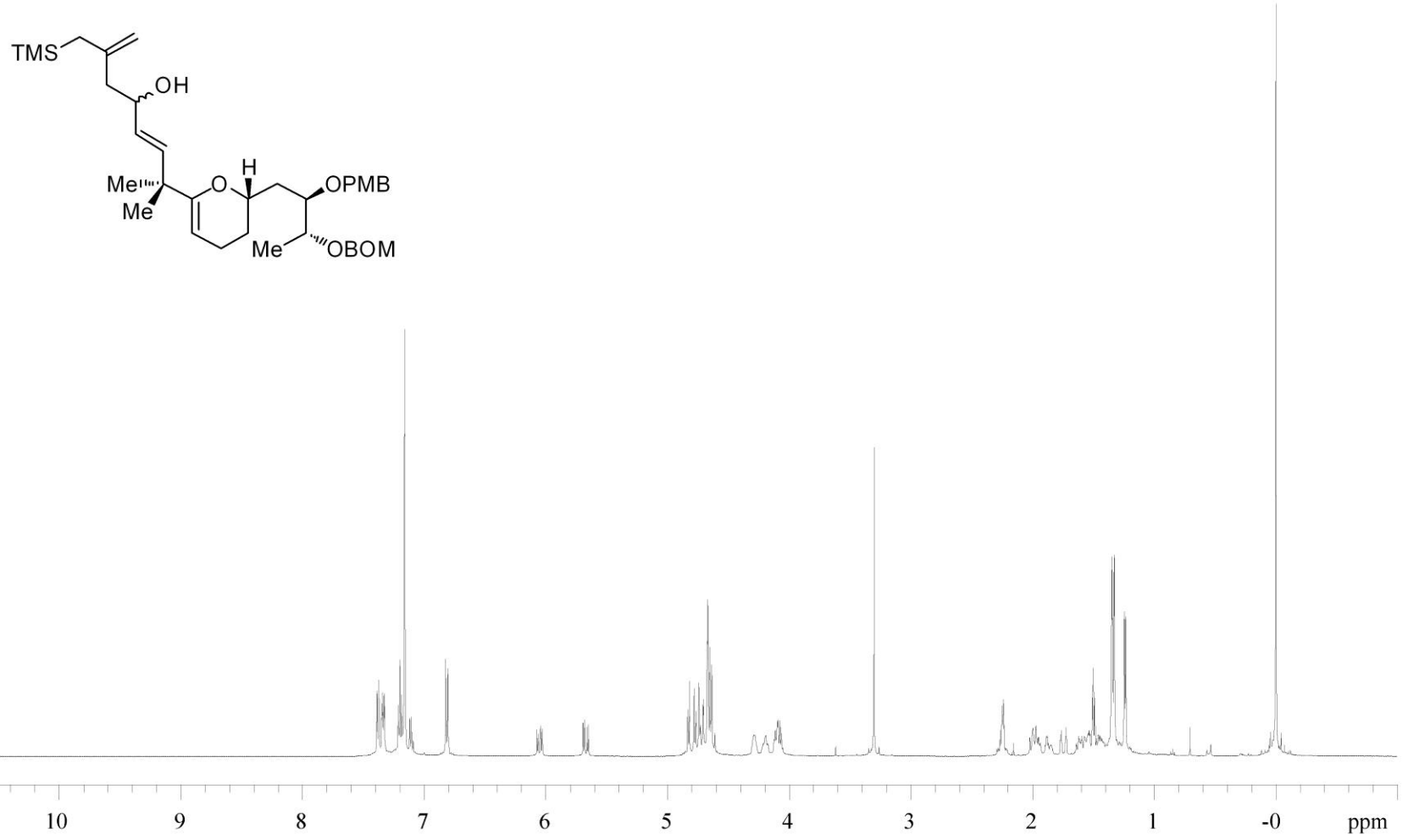

EtS
O
Me
Me
H
O
Me
OPMB
OBOM
ppm
200
180
160
140
120
100
80
60
40
20

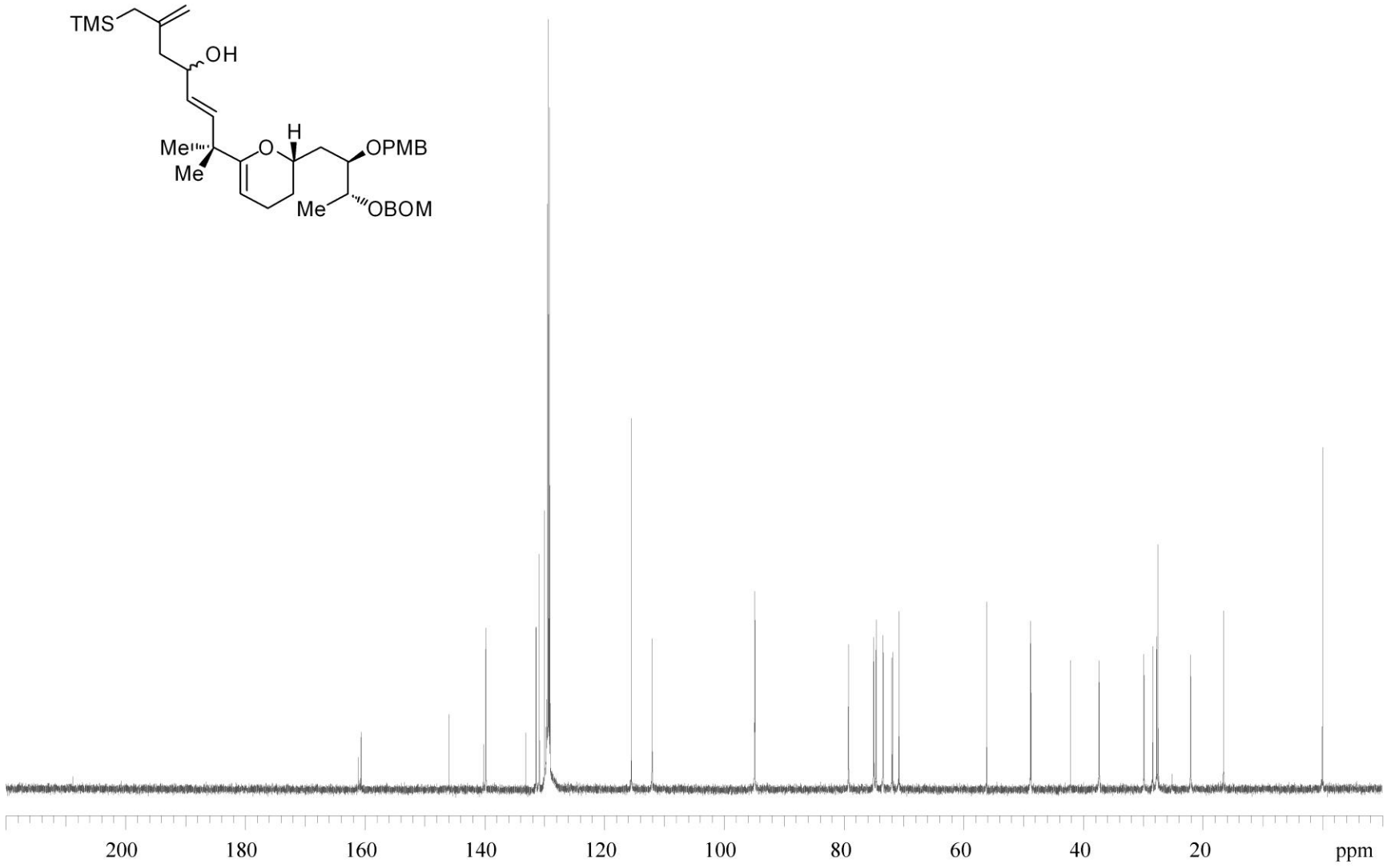

EtS
O
H
Me
Me
O
OPMB
Me
OBOM
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

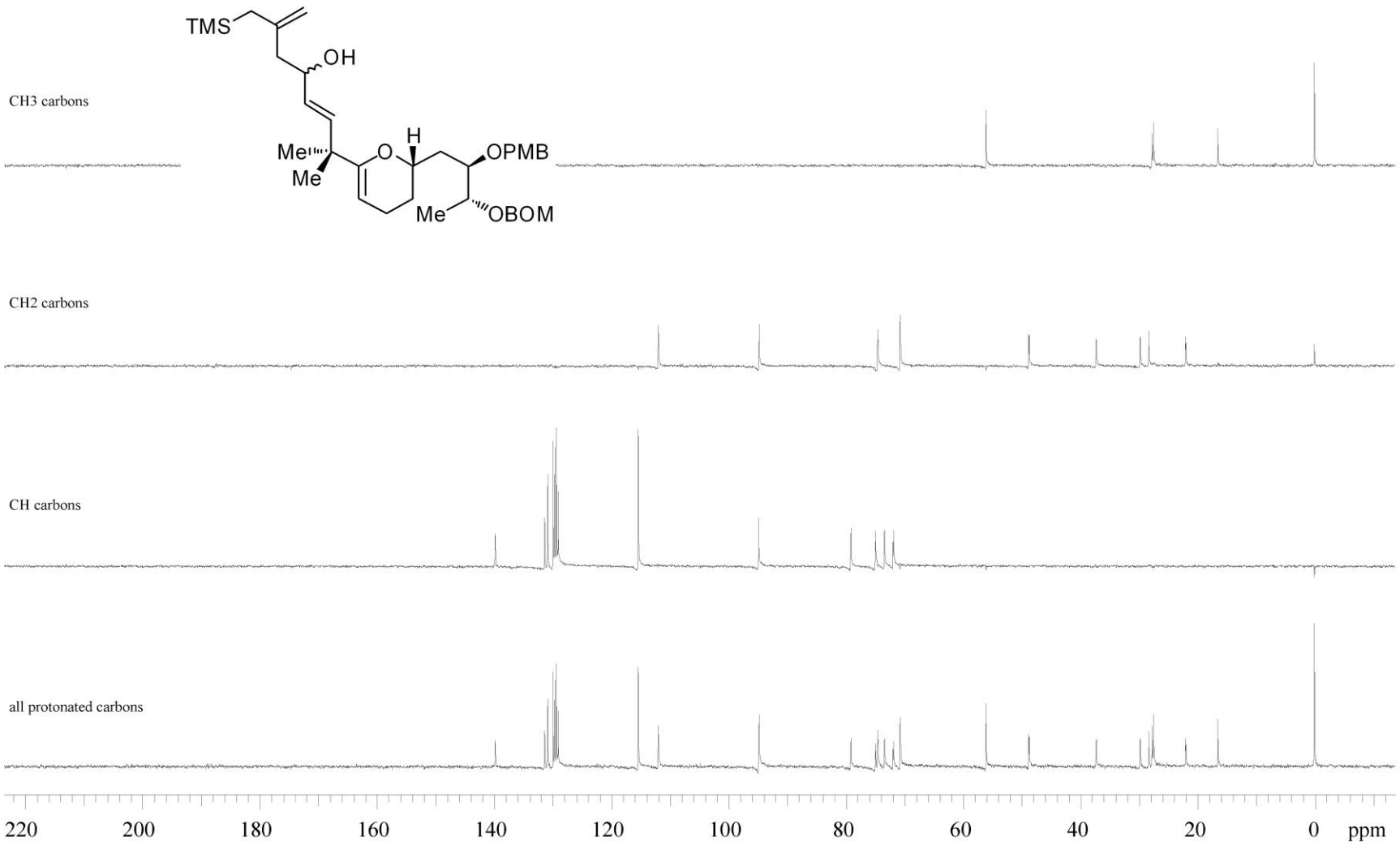

OPMB
OBOM
Me
Me
Me
H
O
H
O
10
9
8
7
6
5
4
3
2
1
-0
ppm

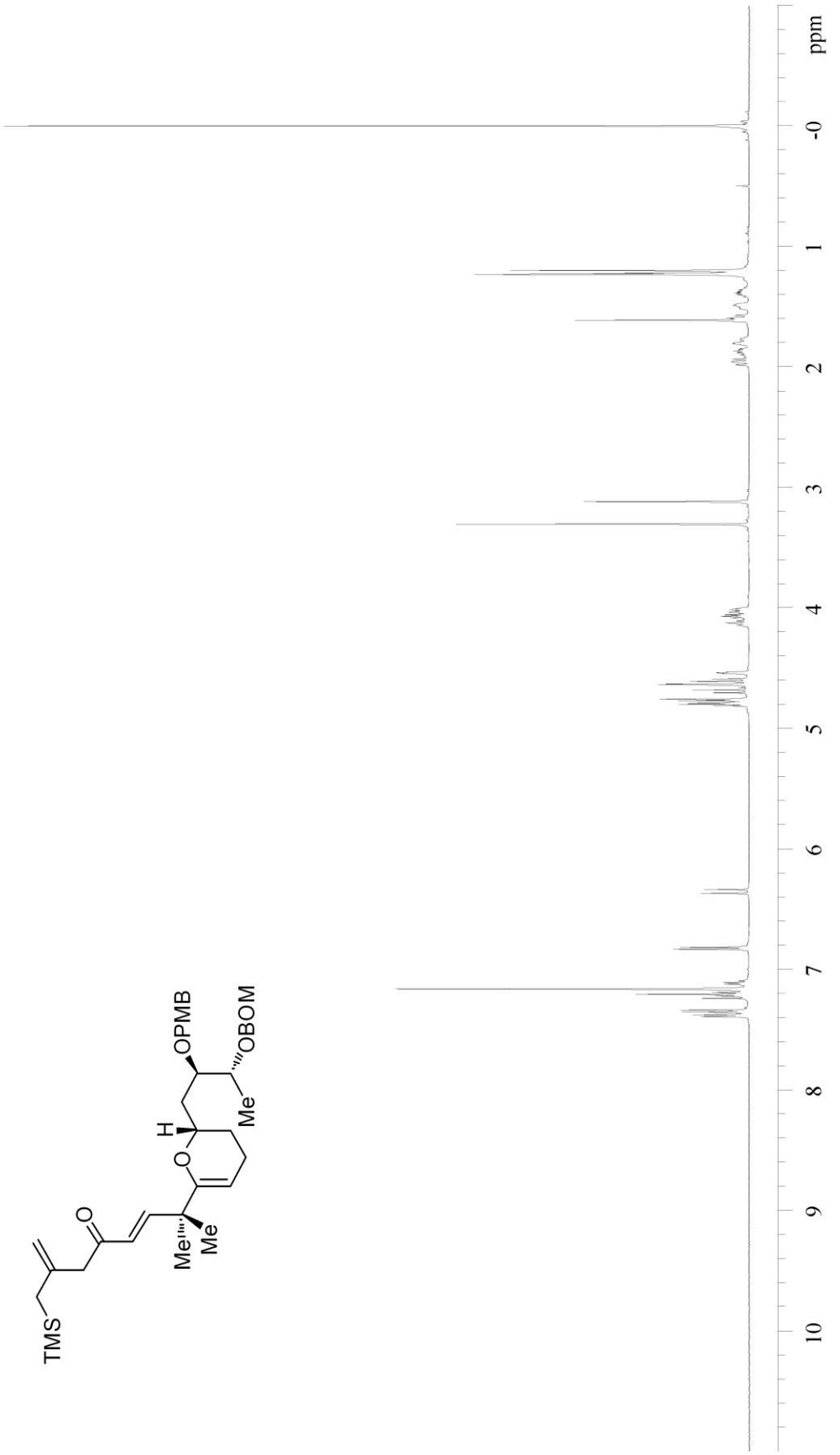

O
H
Me
Me
H
O
OPMB
Me
OBOM
ppm
200
180
160
140
120
100
80
60
40
20

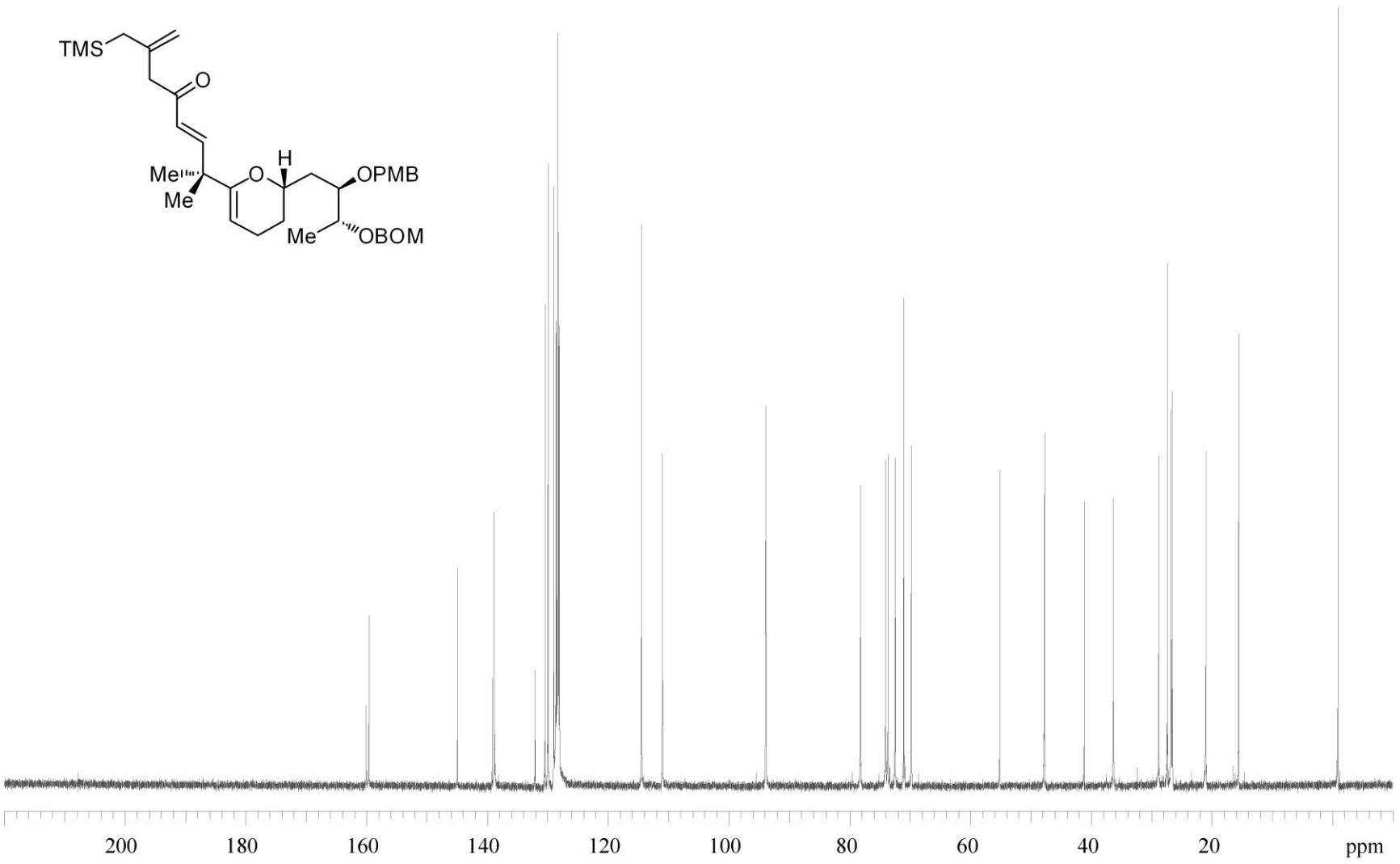

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
OPMB
OBOM
Me
Me
Me
H
O
O
H
200
180
160
140
120
100
80
60
40
20
0
ppm

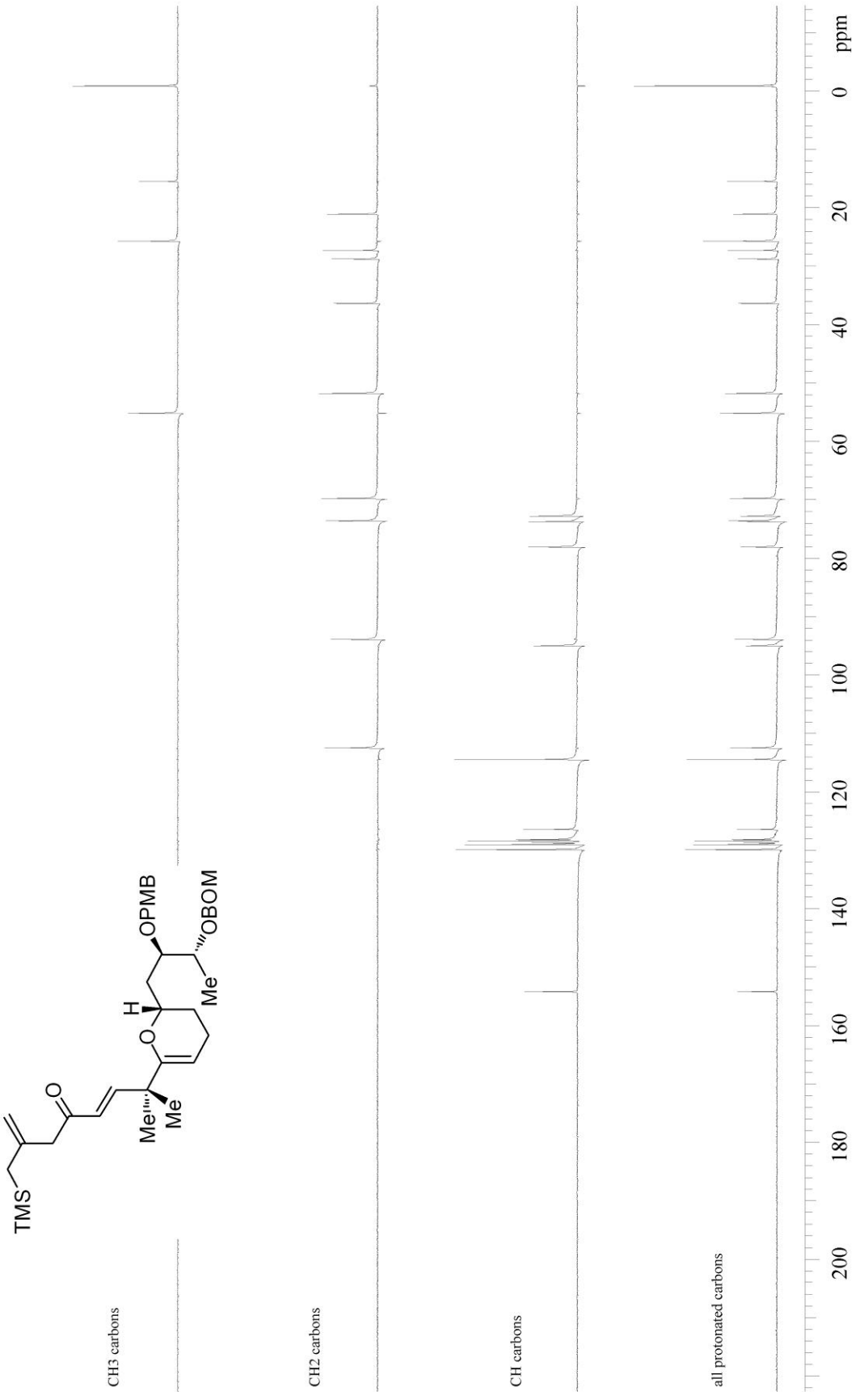

TMS
OH
Me
Me
O
H
OPMB
Me
OBOM
10
9
8
7
6
5
4
3
2
1
-0
ppm

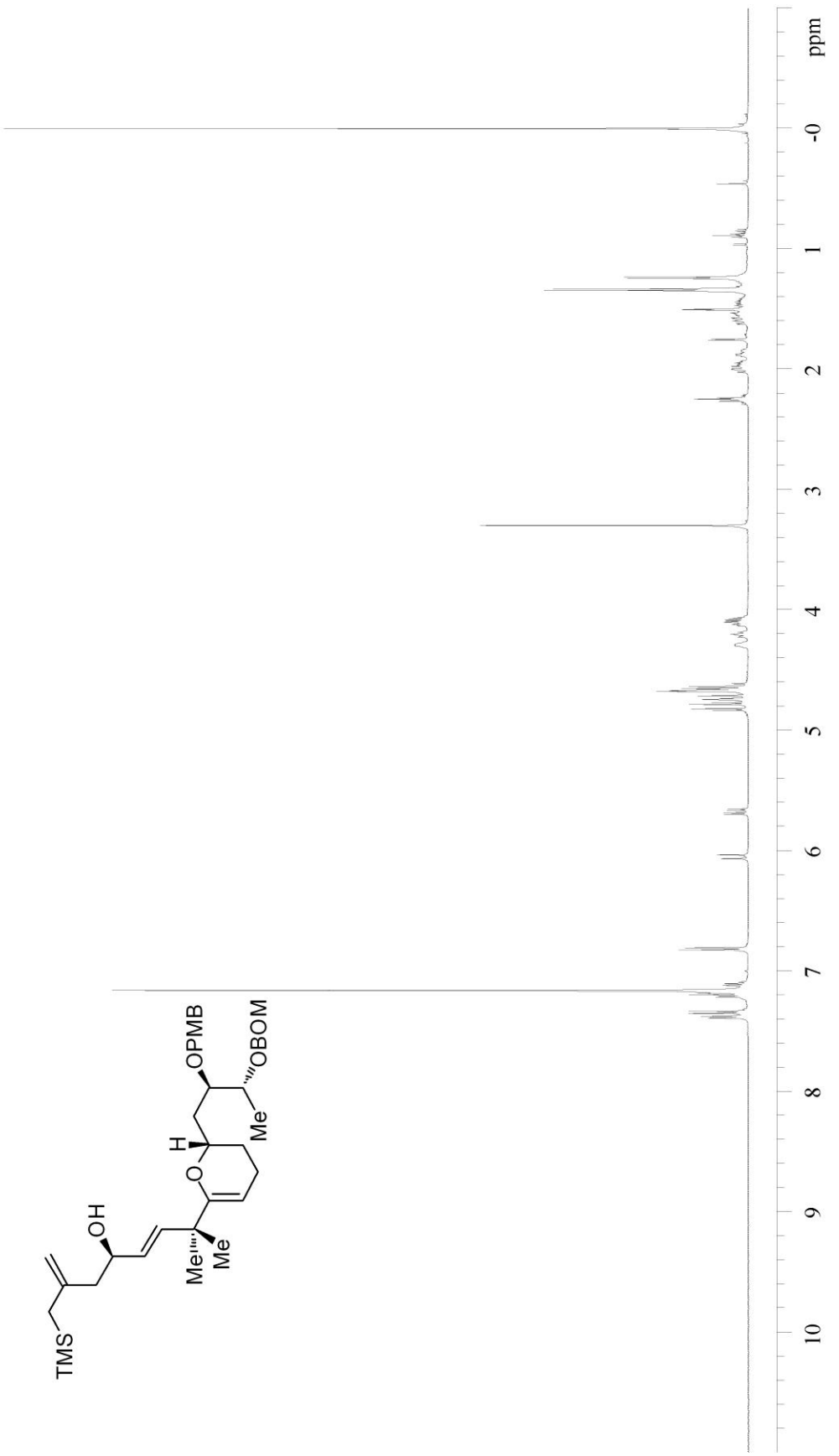

TMS
OH
Me
Me
O
H
OPMB
Me
OBOM
200
180
160
140
120
100
80
60
40
20
ppm

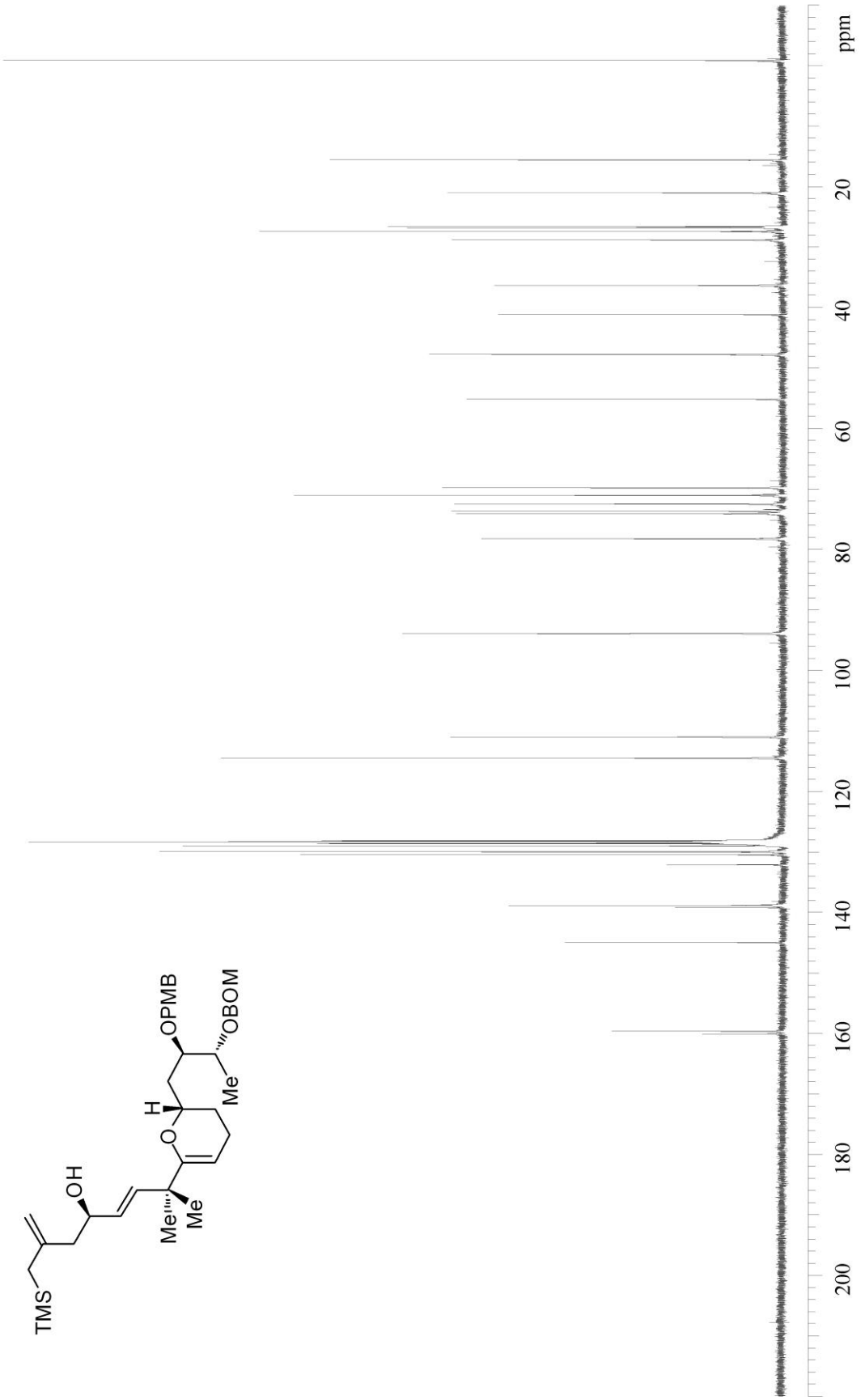

TMS
OH
Me
Me
O
H
OPMB
Me
OBOM
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
220
200
180
160
140
120
100
80
60
40
20
0
ppm

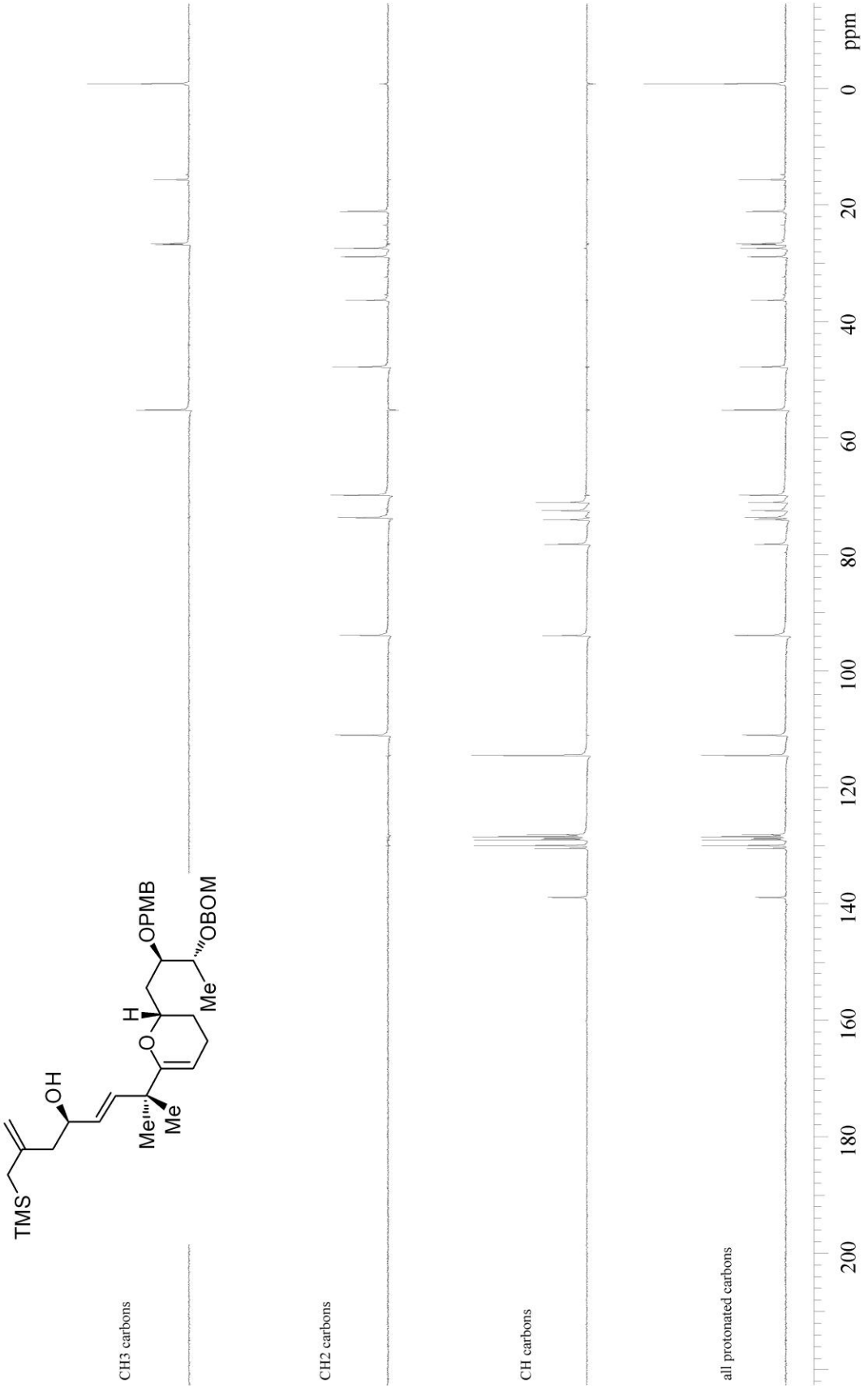

TMS
O
Me
Me
H
O
Me
OPMB
OBOM
10
9
8
7
6
5
4
3
2
1
-0
ppm

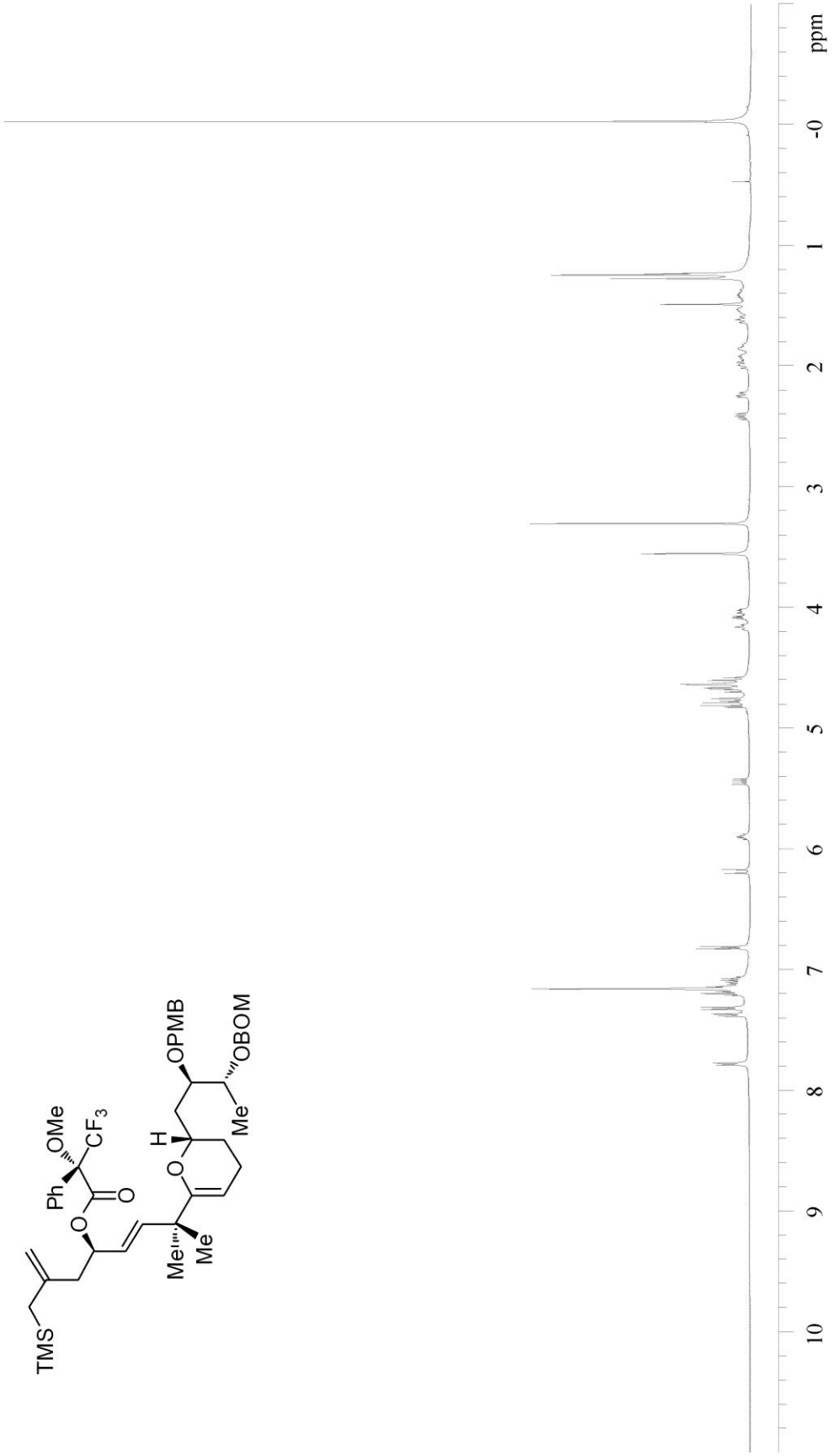

TMS
O
H
Me
Me
O
OPMB
Me
OBOM
200
180
160
140
120
100
80
60
40
20
ppm

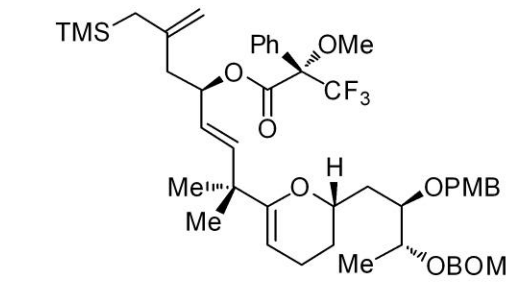

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
TMS
O
Me
Me
H
O
OPMB
OBOM
Me
200
180
160
140
120
100
80
60
40
20
0
ppm

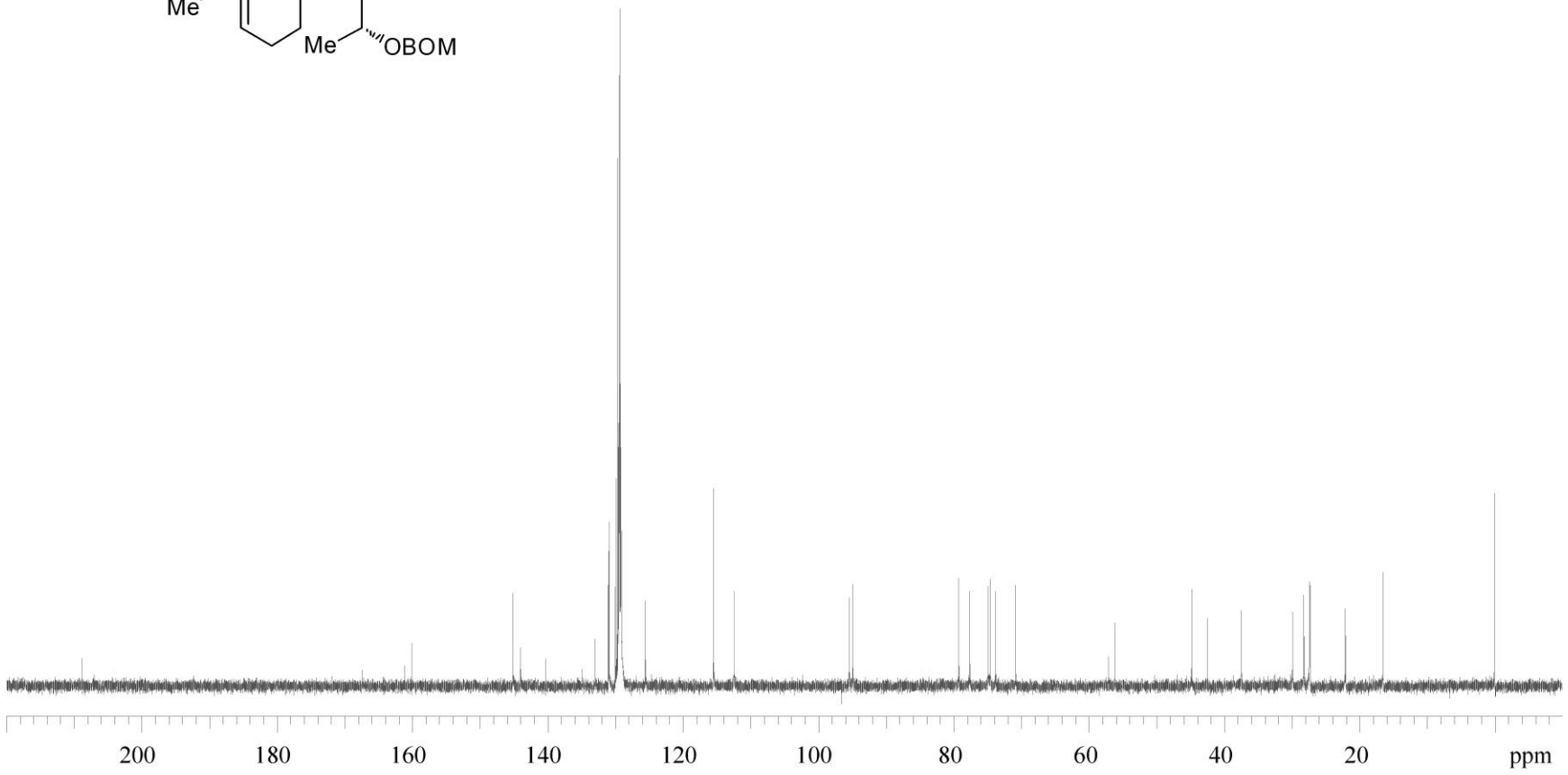

TMS
OH
Me
Me
H
O
OPMB
Me
OBOM
10
9
8
7
6
5
4
3
2
1
-0
ppm

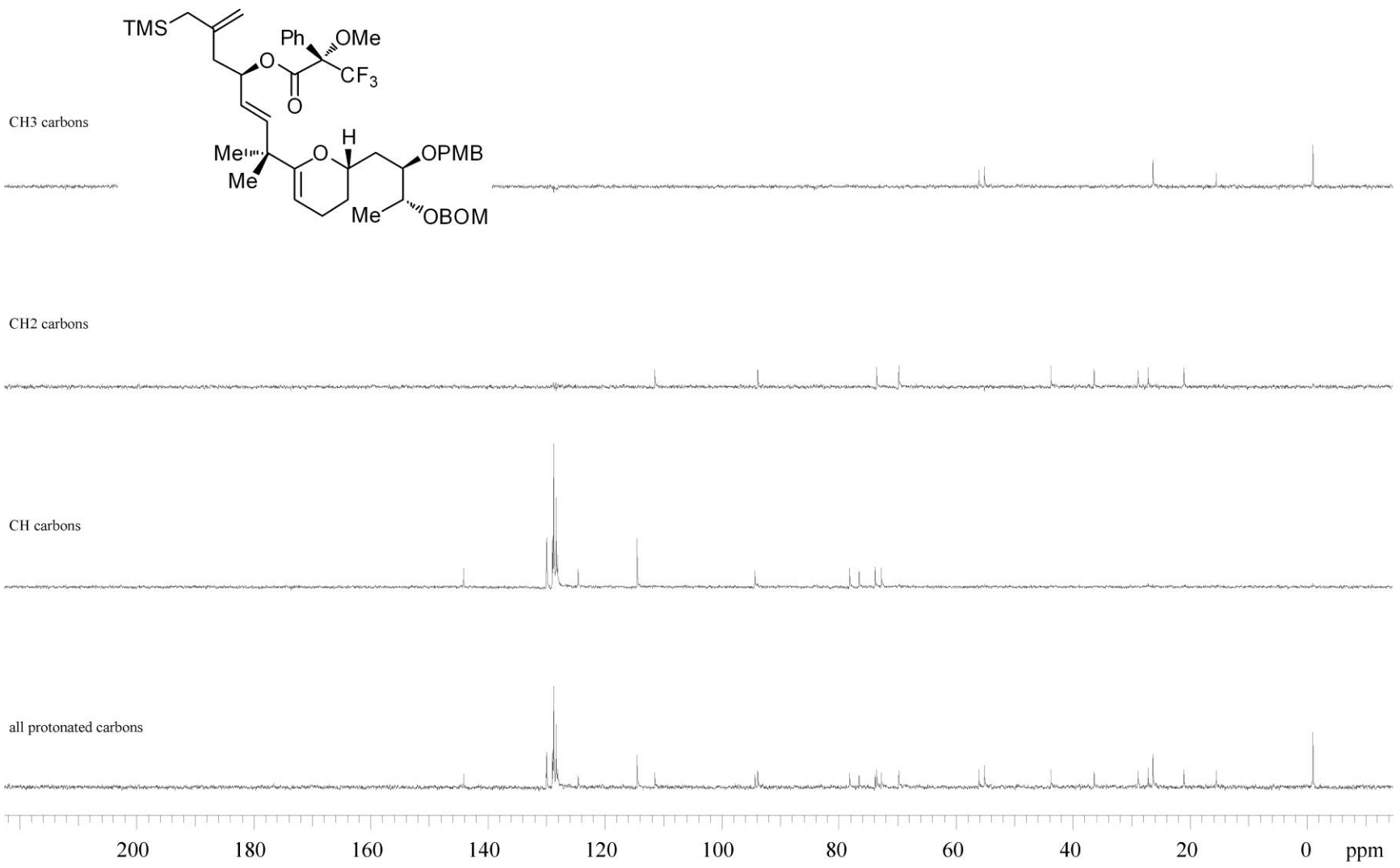

TMS
OH
Me
Me
O
H
Me
OPMB
OBOM
ppm
200
180
160
140
120
100
80
60
40
20

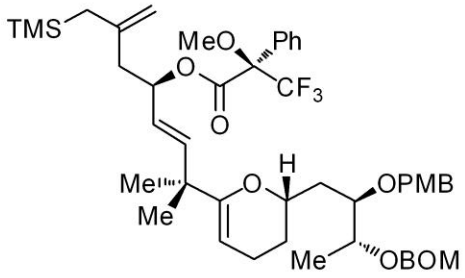

TMS
OH
Me
Me
H
O
OPMB
Me
OBOM
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm


TMS
Ph
OMe
CF3
O
O
O
H
Me
Me
OPMB
Me
OBOM
10
9
8
7
6
5
4
3
2
1
-0
ppm

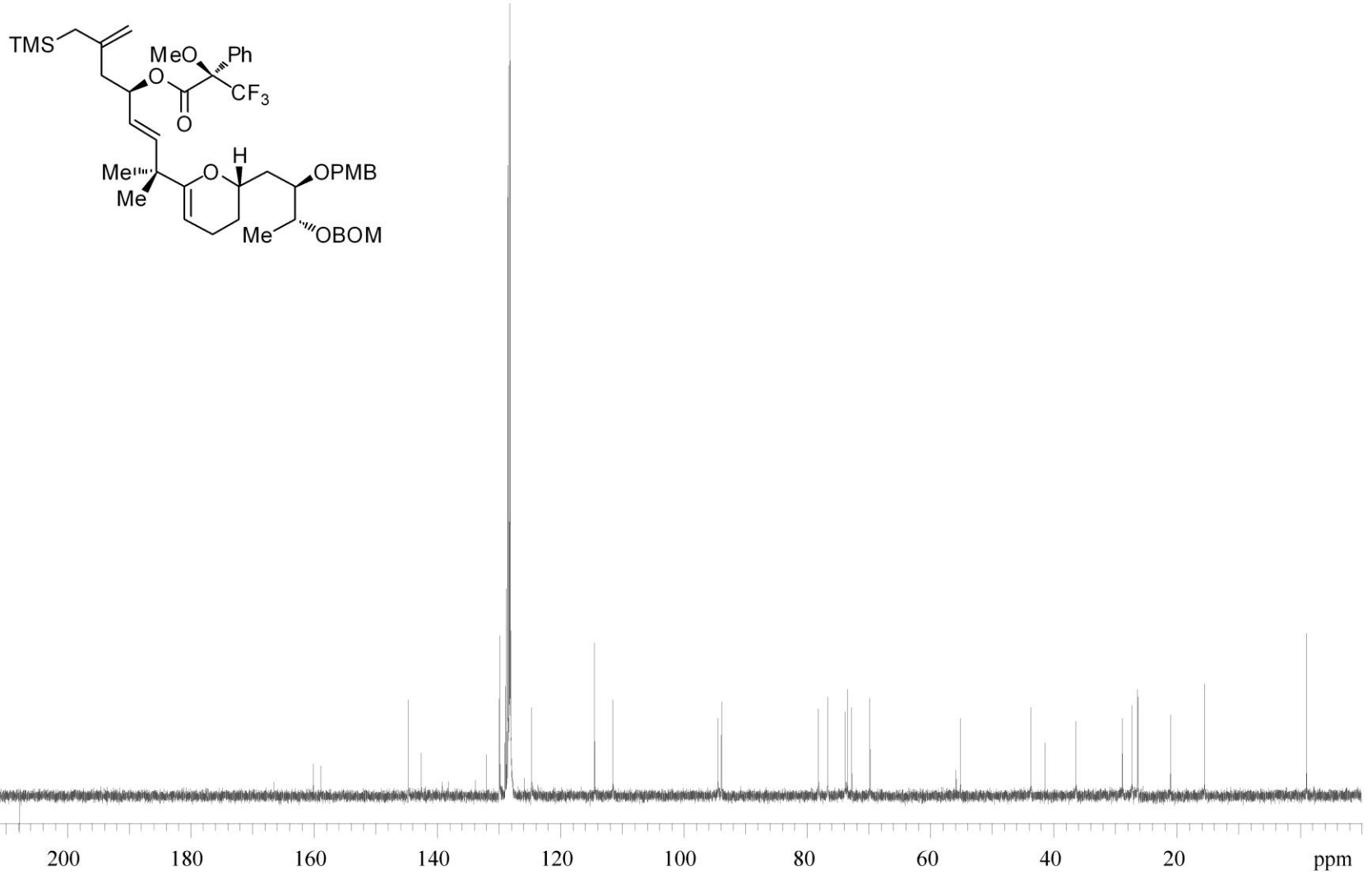

TMS
Ph
OMe
O
CF3
O
H
Me
Me
O
OPMB
Me
OBOM

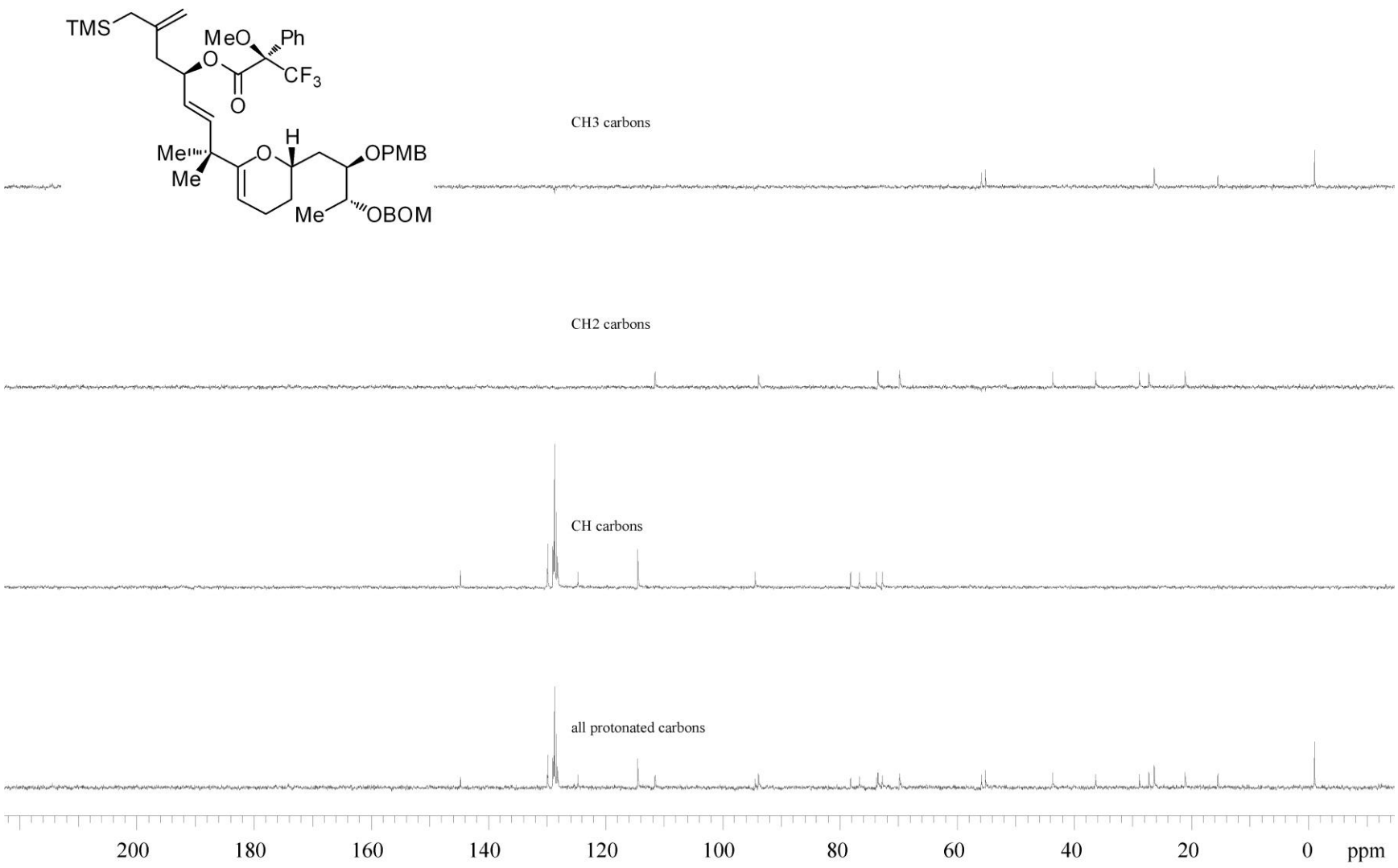

200
180
160
140
120
100
80
60
40
20
ppm

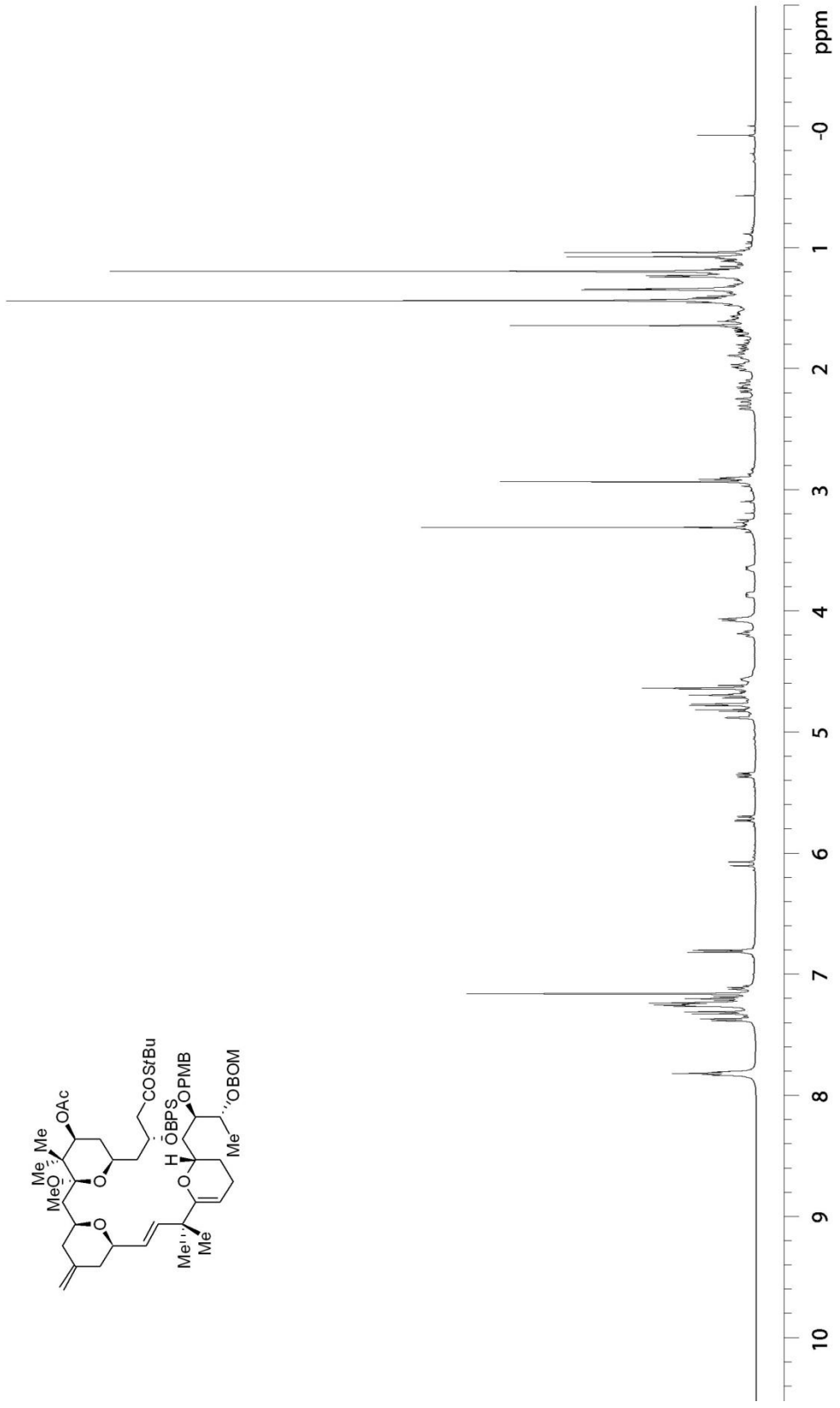

TMS
Ph
OMe
O
CF3
O
CH3 carbons
Me
Me
O
H
OPMB
Me
OBOM
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

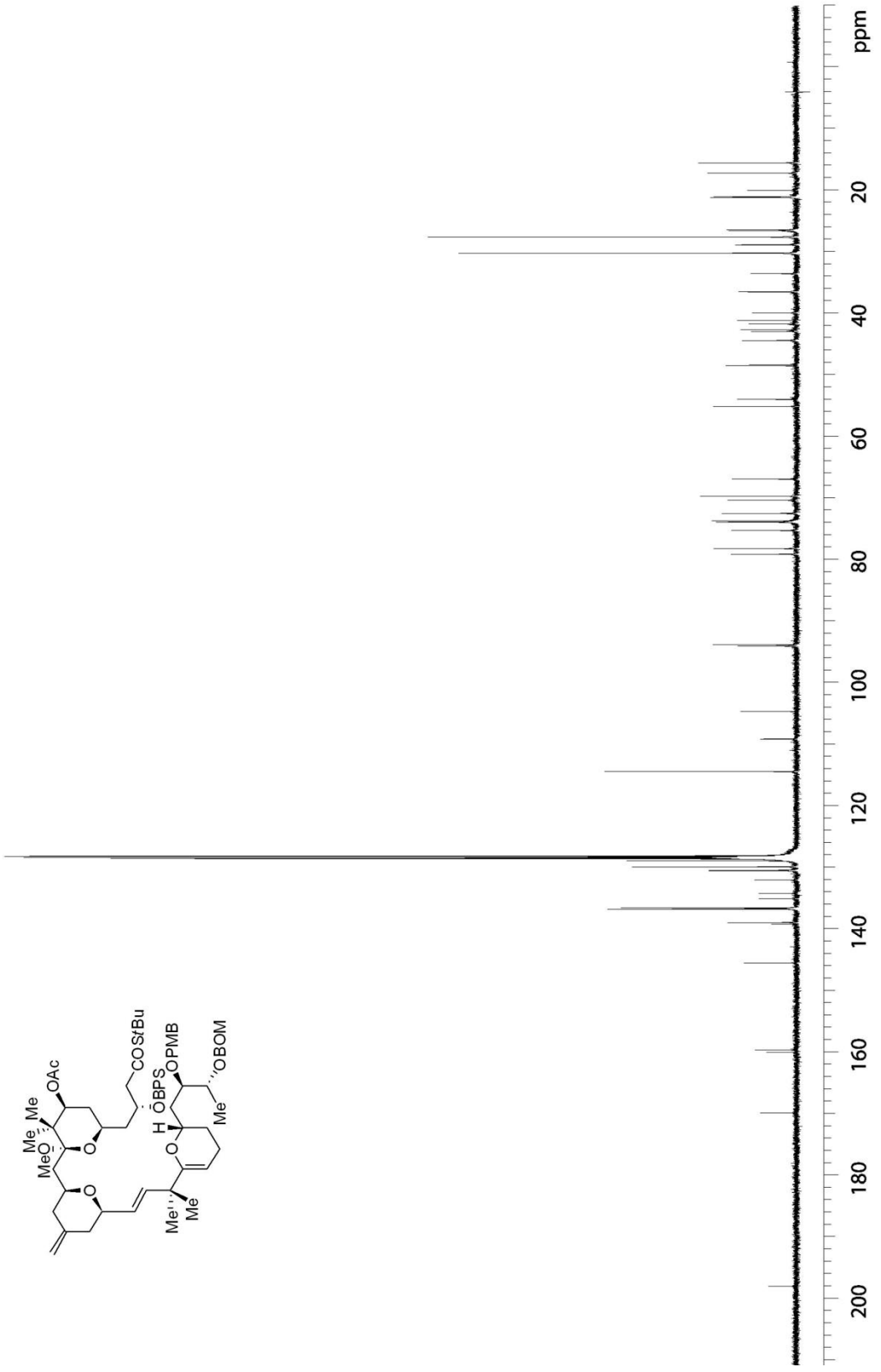

TMS
MeO
Ph
O
CF3
O
H
Me
Me
O
OPMB
Me
OBOM

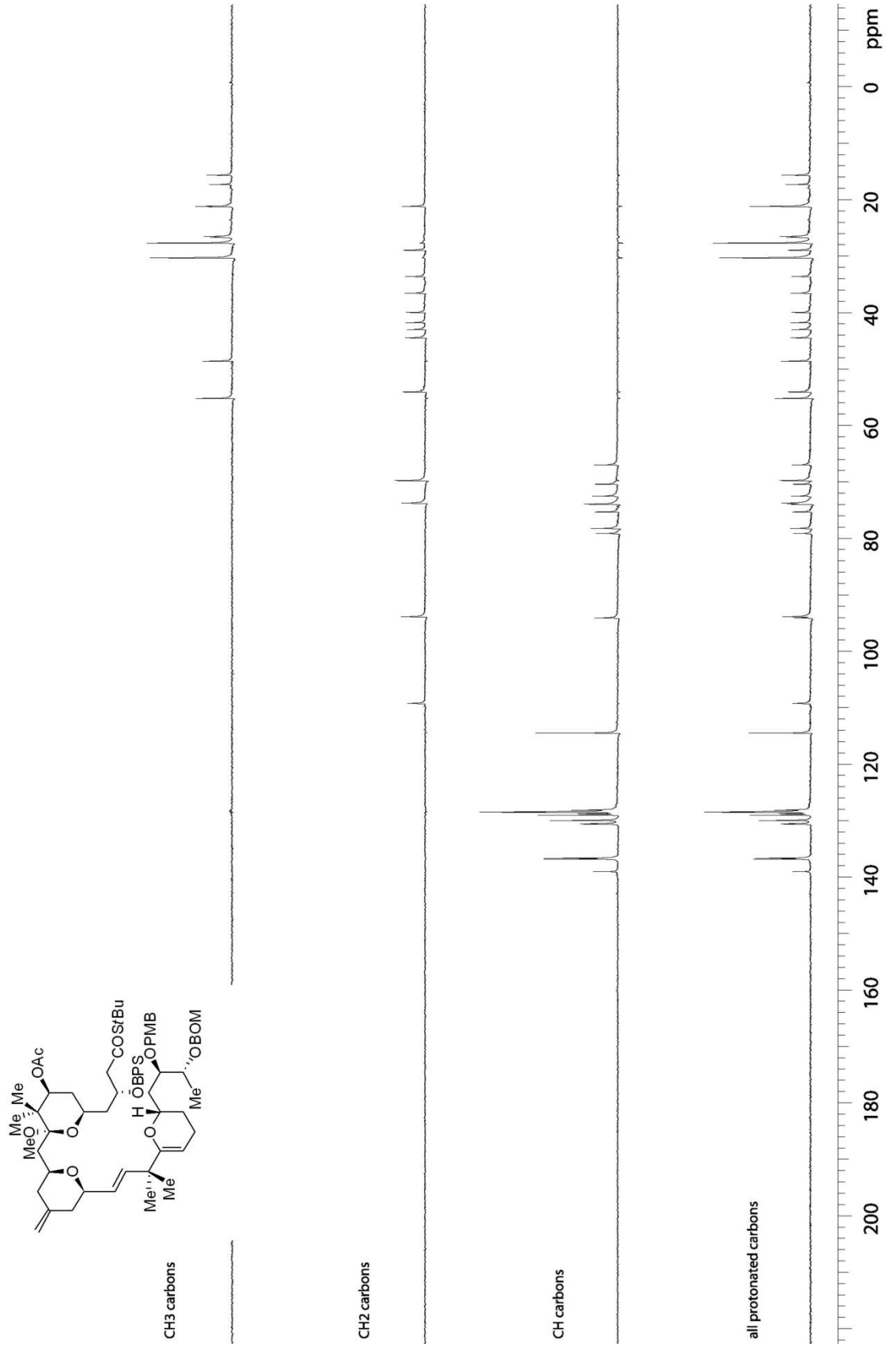
10
9
8
7
6
5
4
3
2
1
-0
ppm

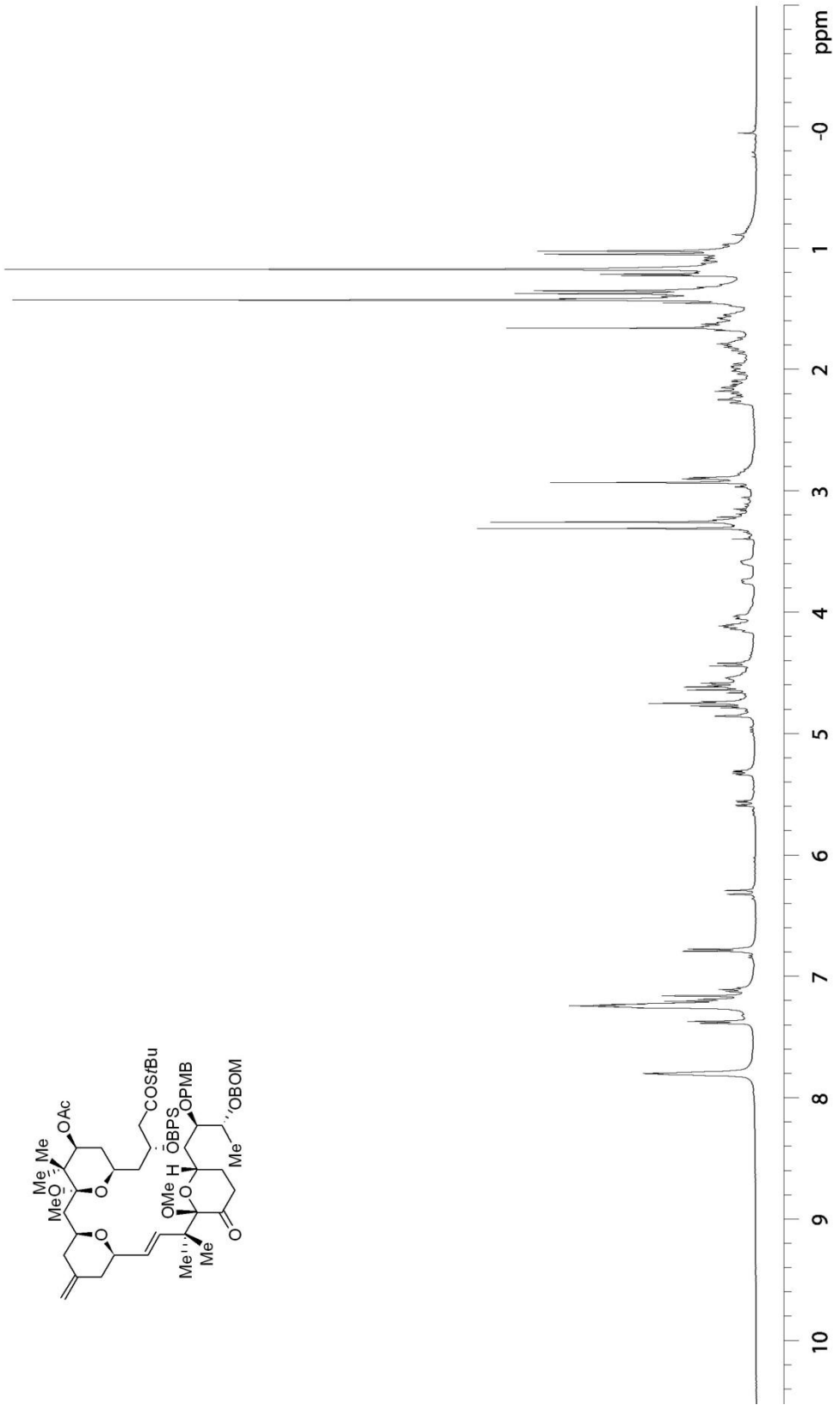

TMS
MeO
Ph
O
CF3
O
H
Me
Me
O
OPMB
Me
OBOM
200
180
160
140
120
100
80
60
40
20
ppm

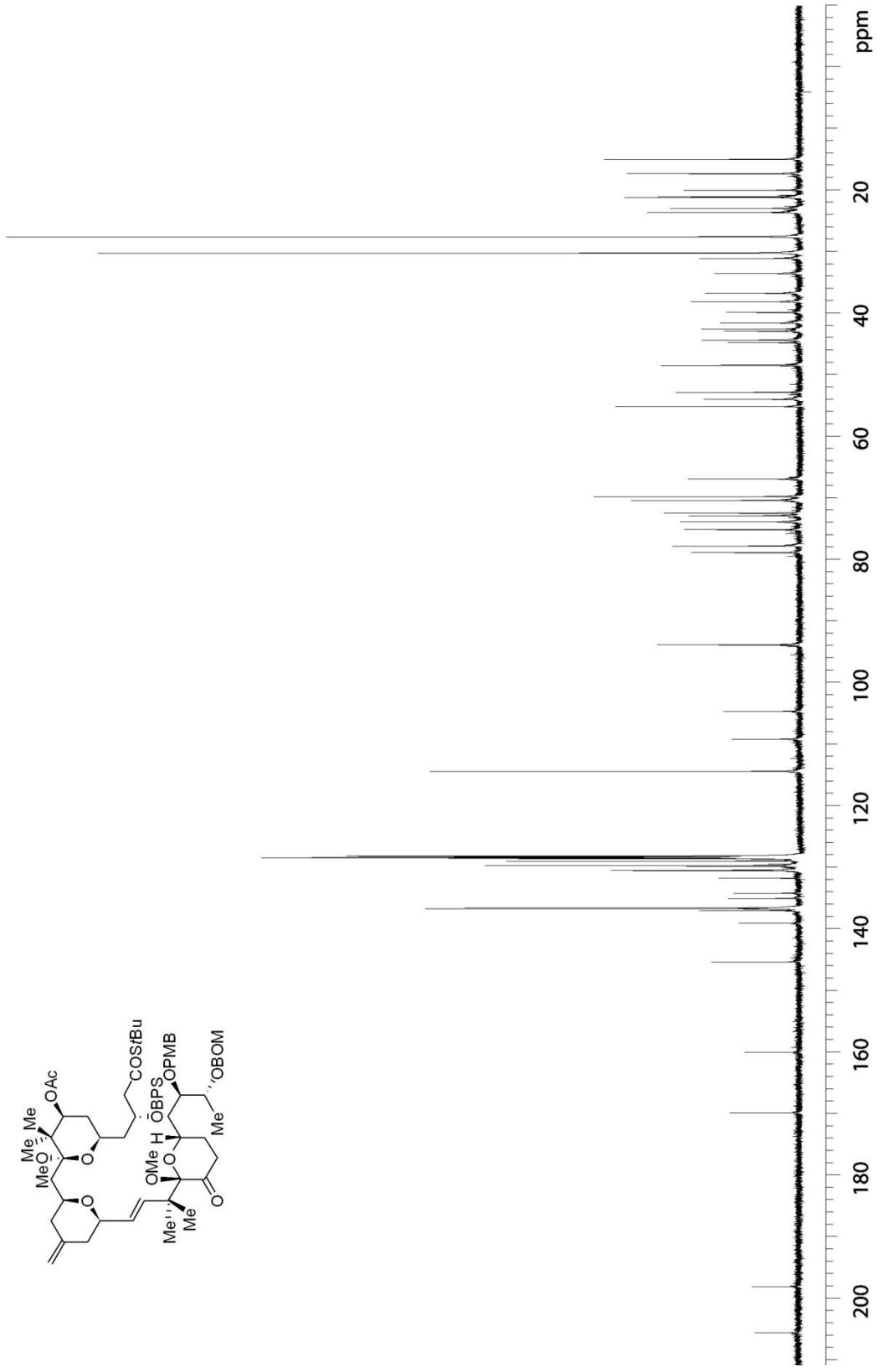

TMS
MeO
Ph
O
CF3
O
H
Me
Me
O
OPMB
Me
OBOM
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

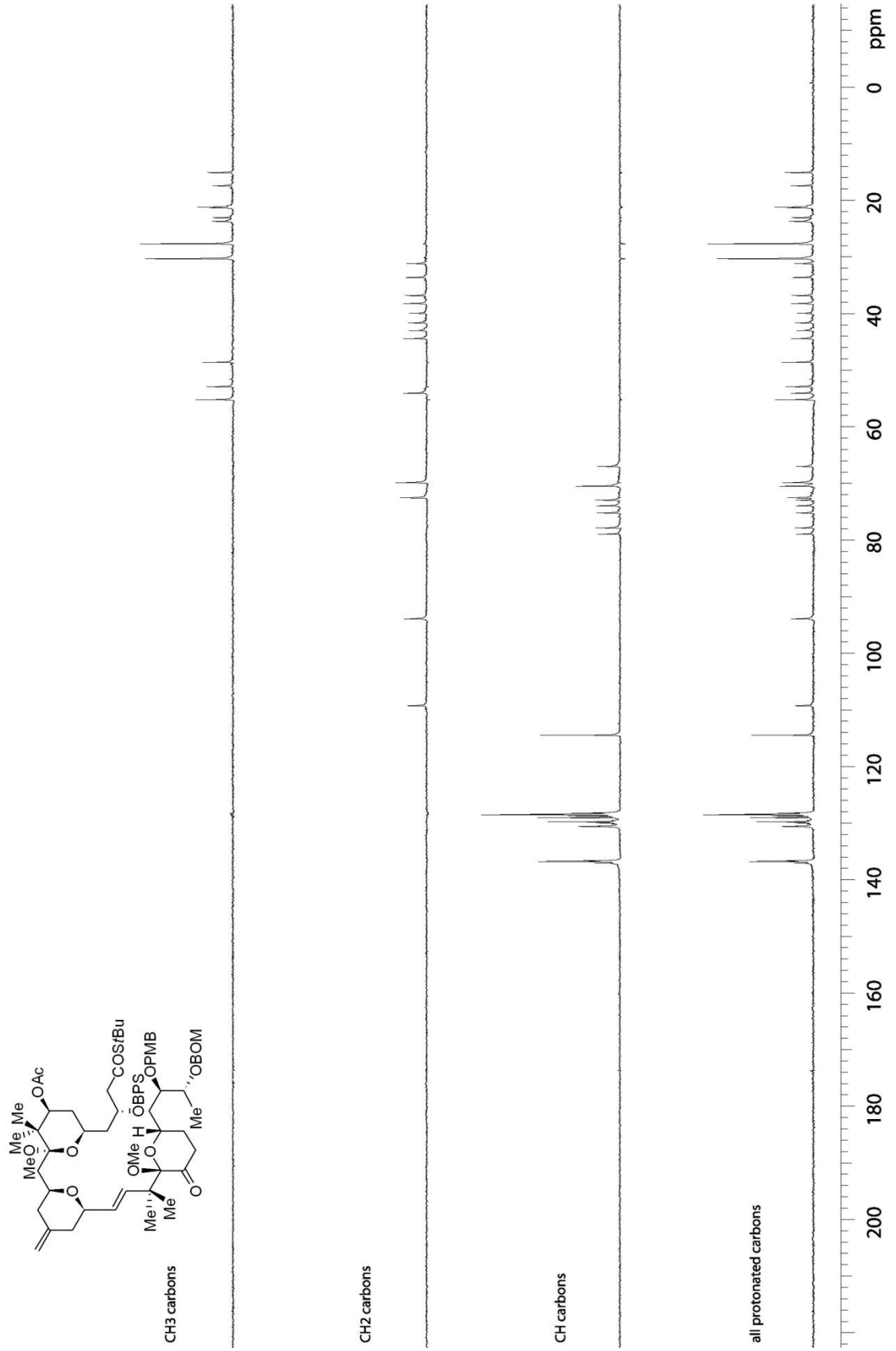

OAc
Me
Me
MeO
O
O
COStBu
H
OBPS
OPMB
O
Me
Me
Me
OBOM
ppm
10
9
8
7
6
5
4
3
2
1
-0

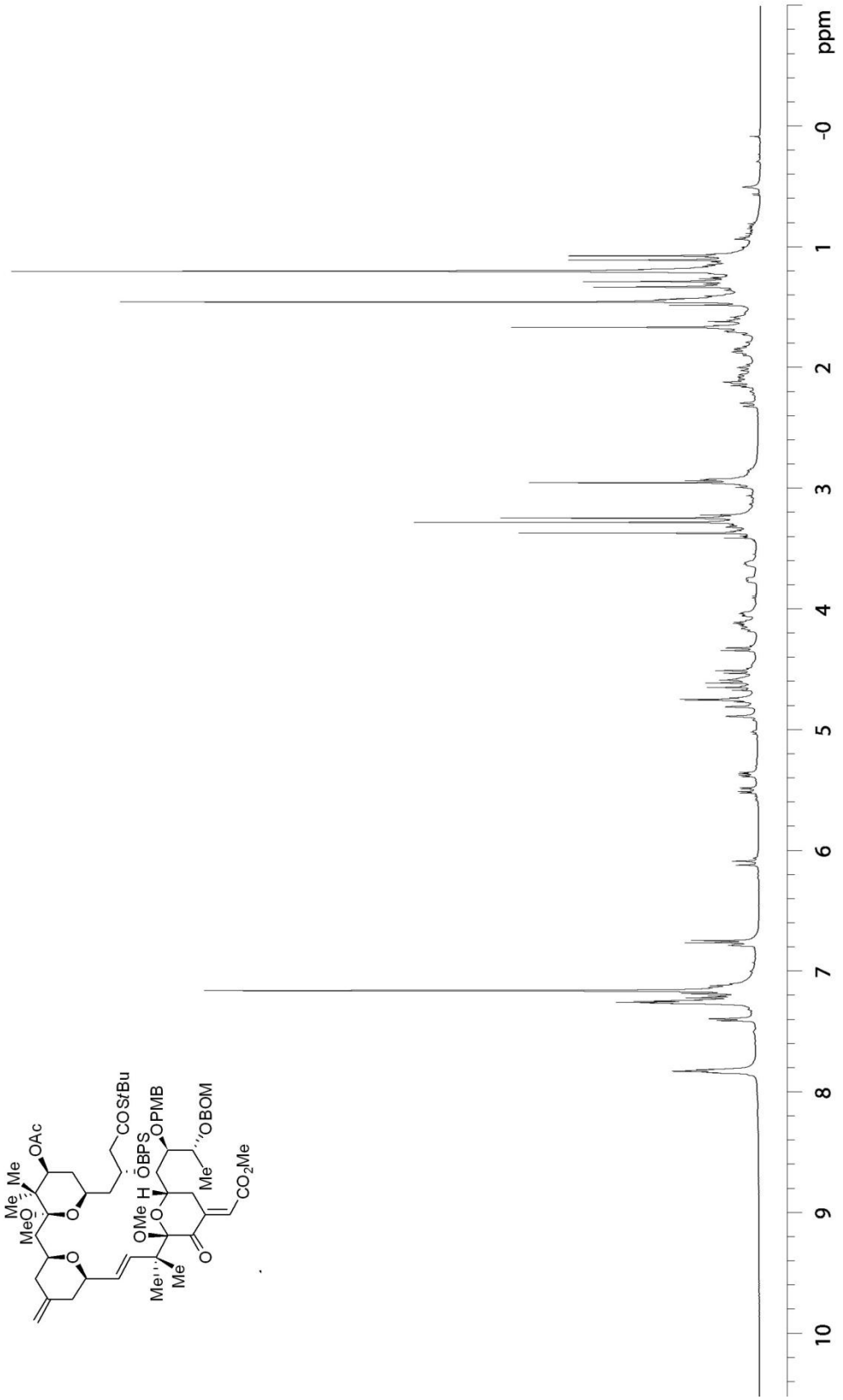

OAc
Me
Me
MeO
O
O
COStBu
H
OBPS
OPMB
O
Me
OBOM
Me
Me
ppm
200
180
160
140
120
100
80
60
40
20

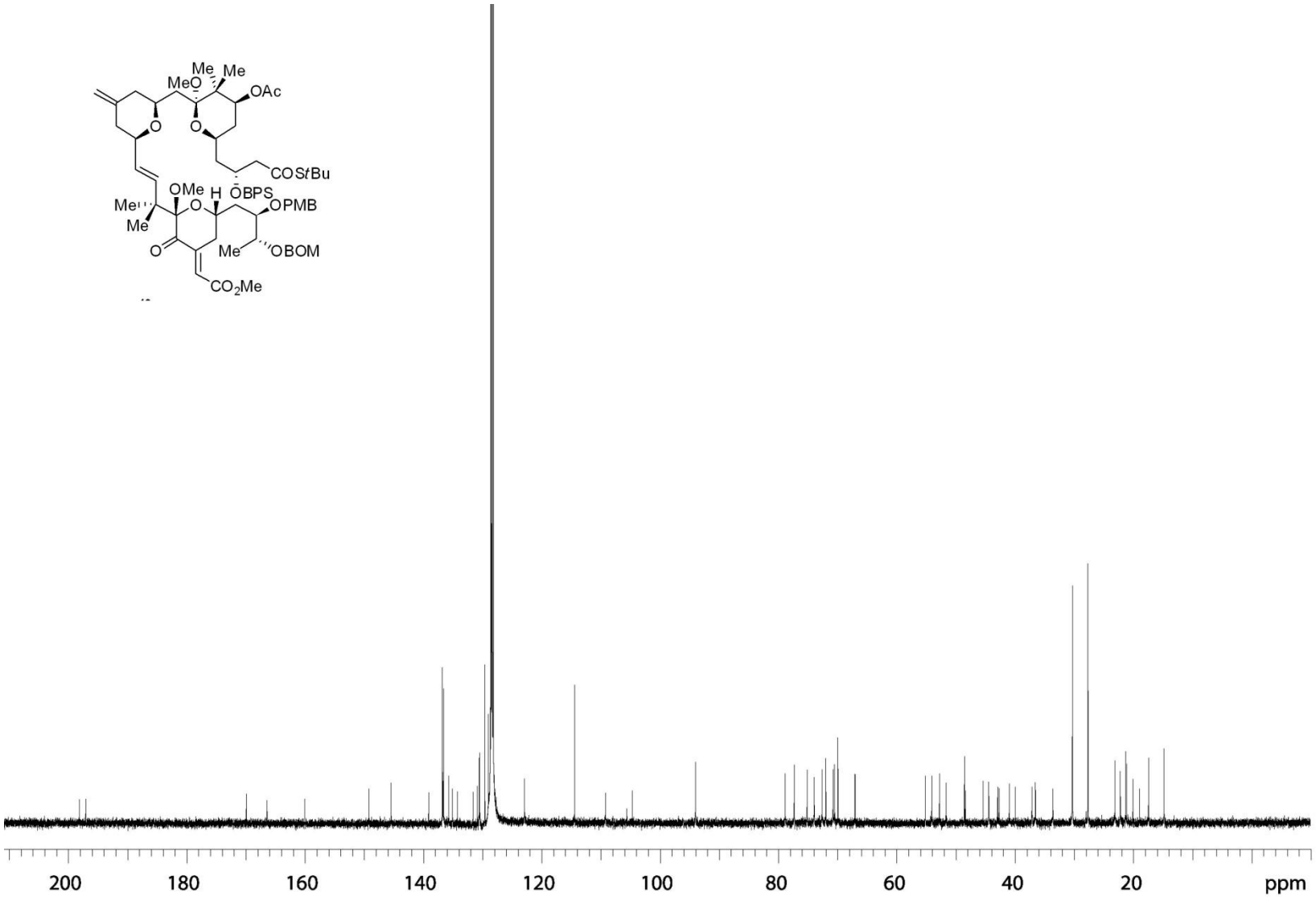

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

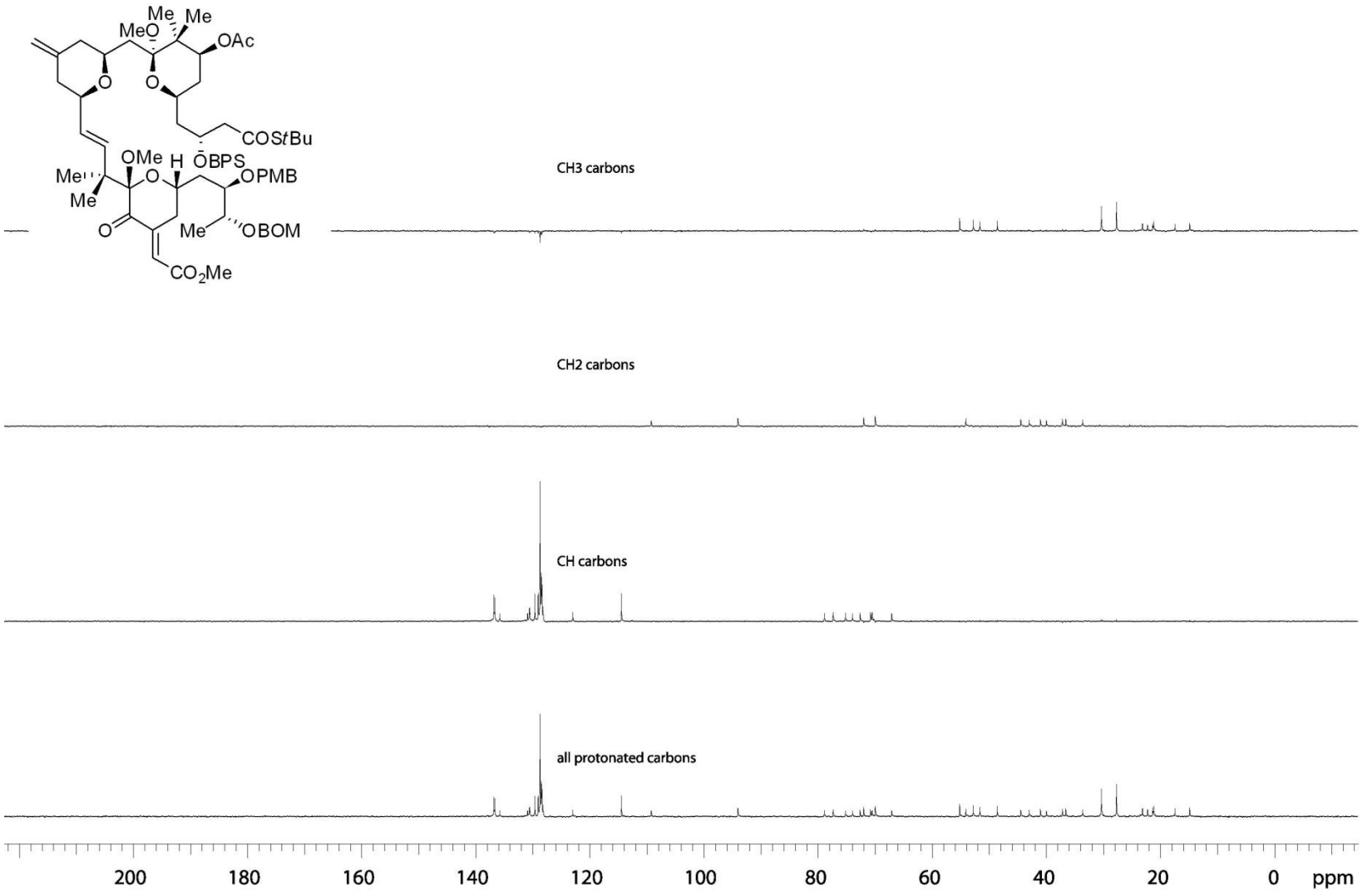

Me
Me
MeO
OAc
O
O
COStBu
OBPS
H
OMe
O
OPMB
Me
OBOM
Me
Me
O
ppm
10
9
8
7
6
5
4
3
2
1
-0

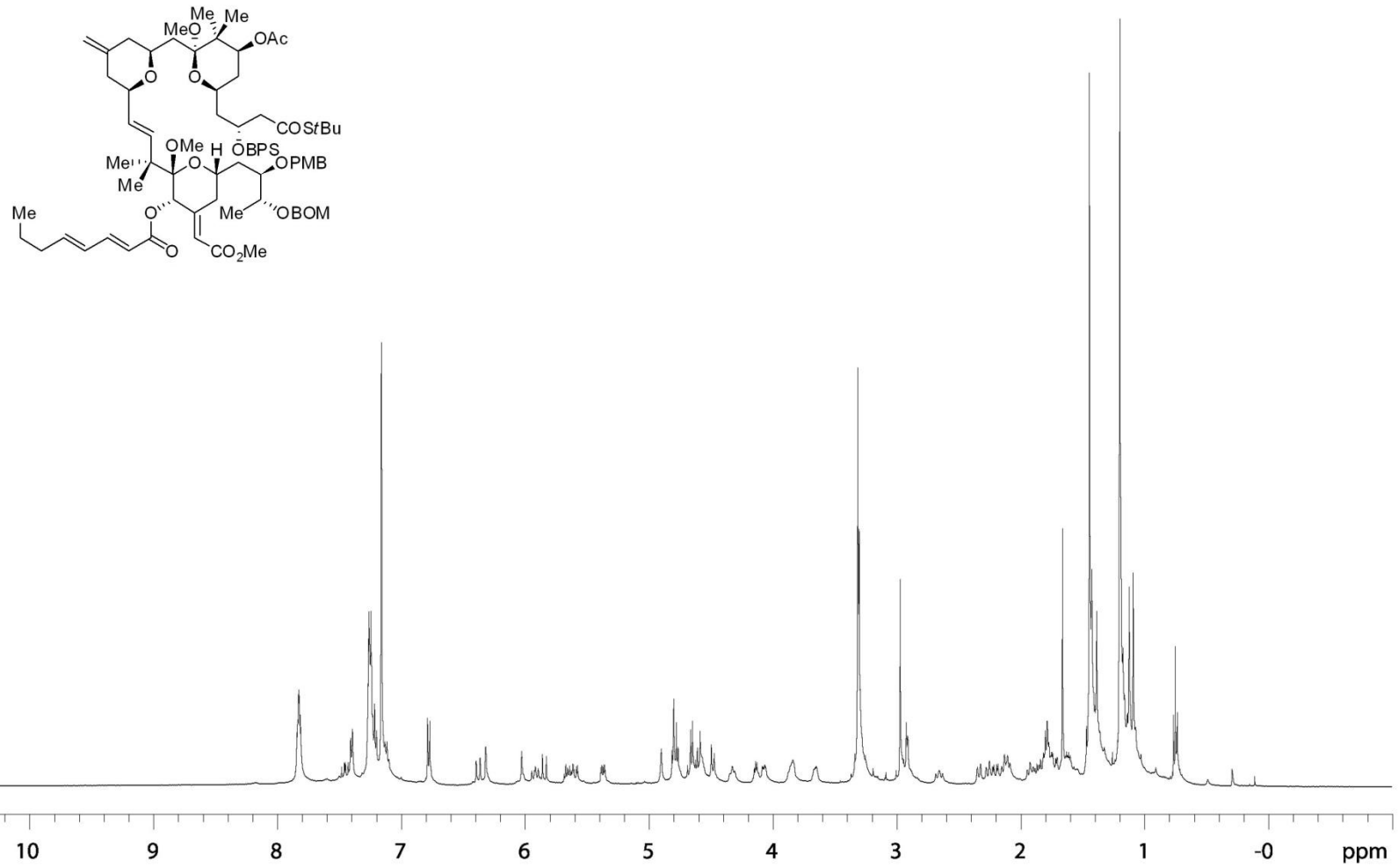

OAc
COS*t*Bu
OPMB
OBOM
OMe
OBPS
Me
Me
Me
Me
Me
O
O
O
O
O
O
ppm
20
40
60
80
100
120
140
160
180
200

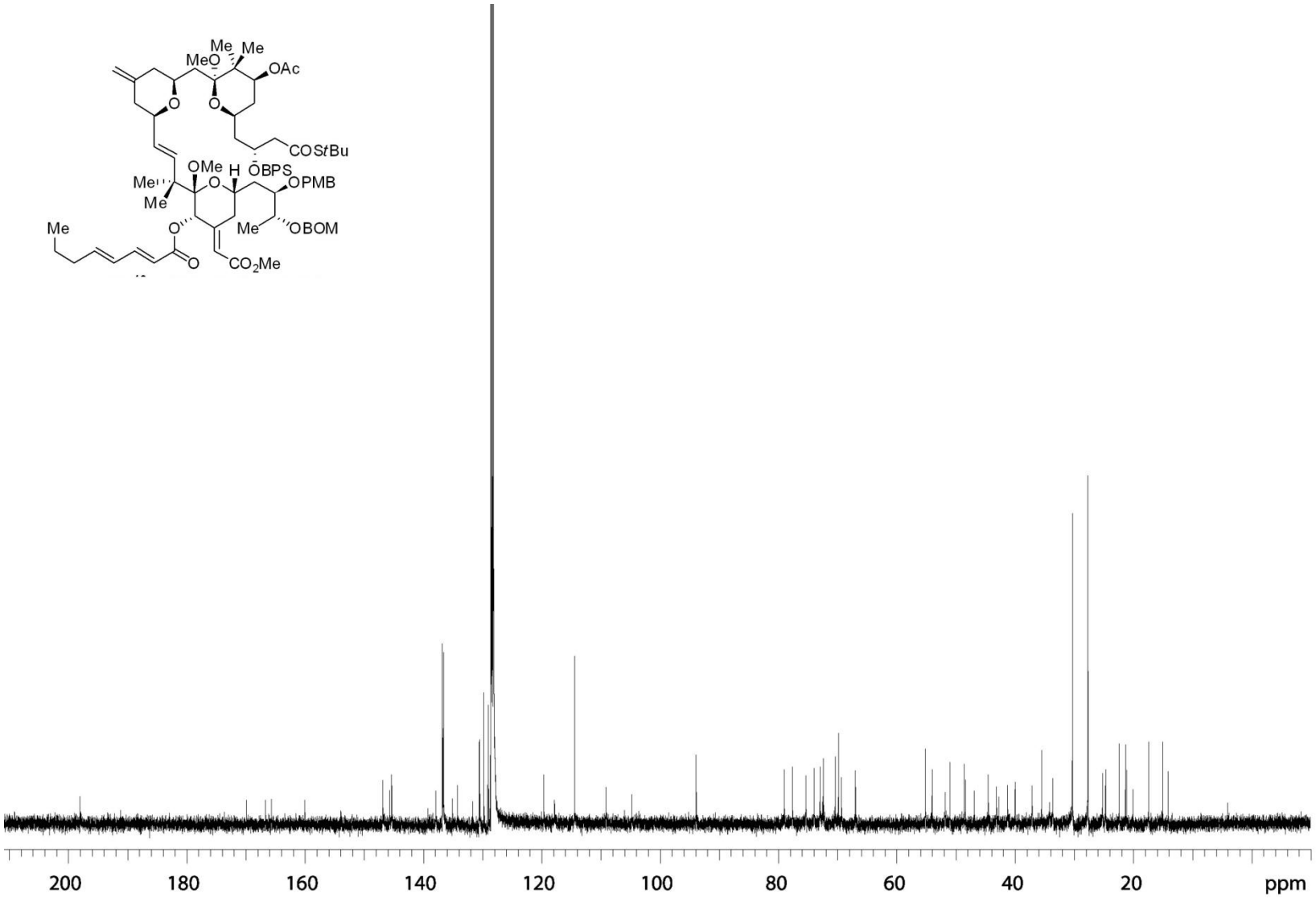

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

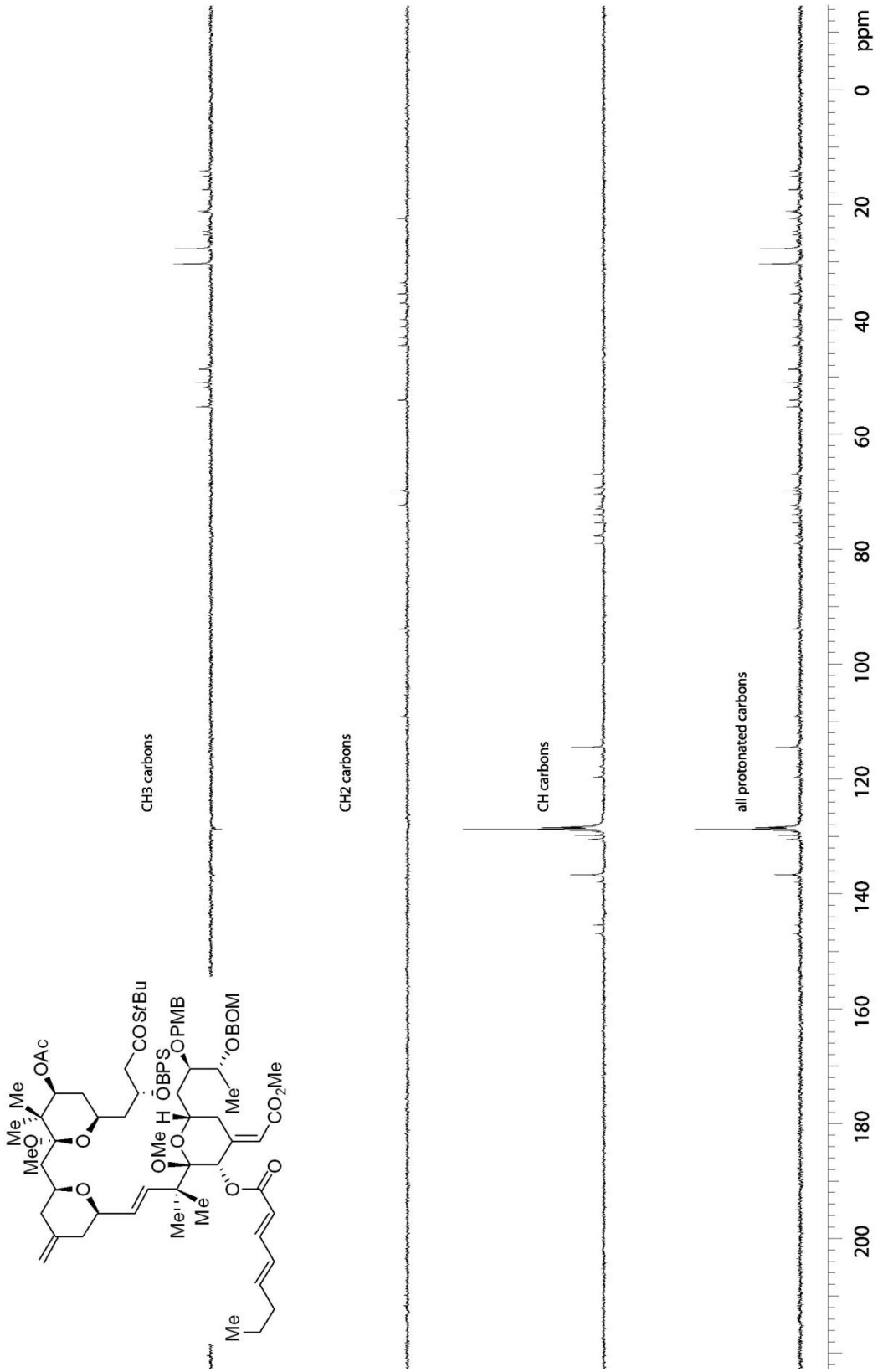

OAc
COStBu
OBPS
OPMB
OBOM
CO2Me
OMe
H
Me
MeO
ppm
10
9
8
7
6
5
4
3
2
1
-0

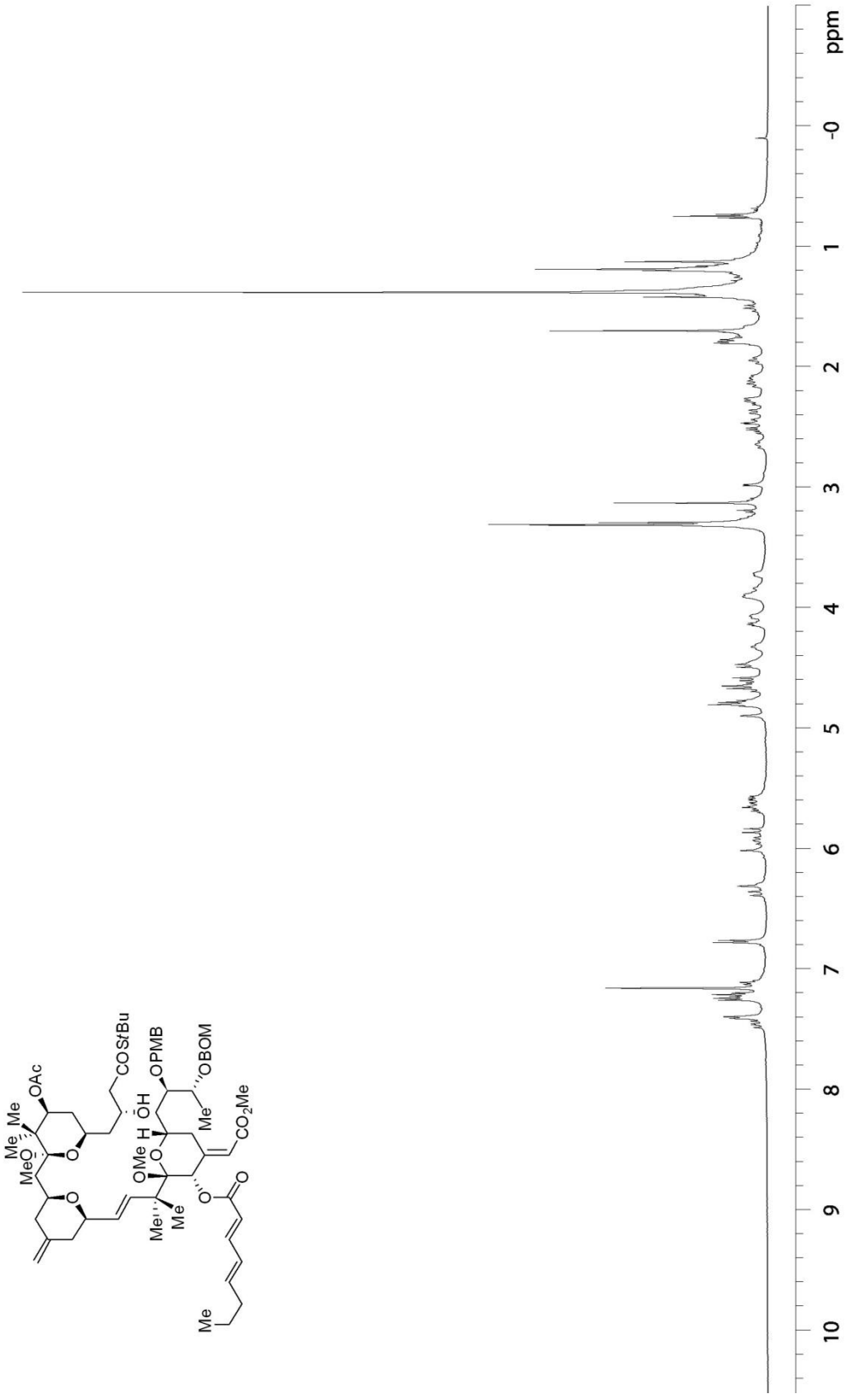

Me
Me
MeO
OAc
O
O
COStBu
OMe
H
OBPS
OPMB
Me
Me
O
Me
OBOM
O
CO2Me
200
180
160
140
120
100
80
60
40
20
ppm

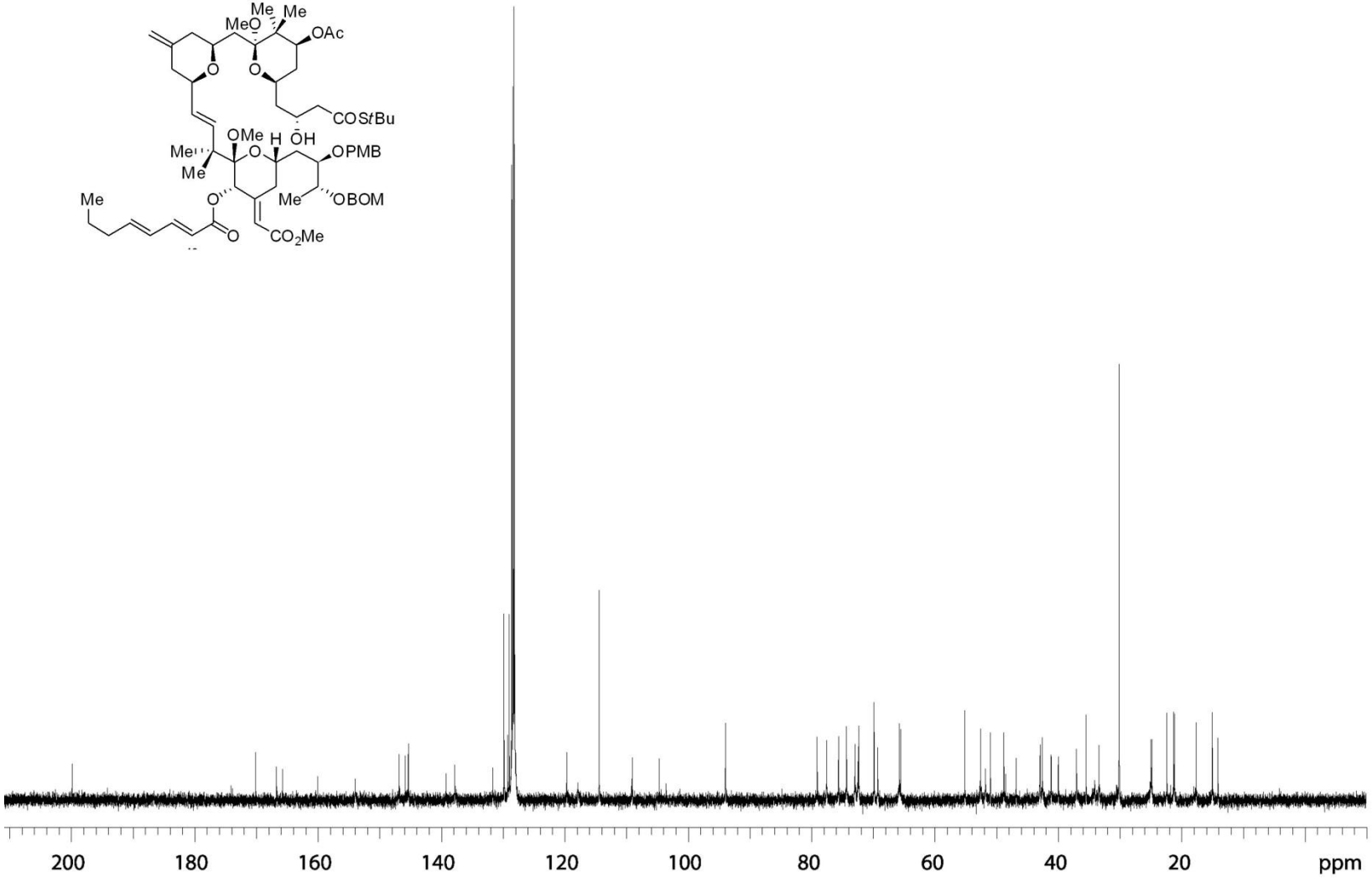

Me
Me
MeO
OAc
O
O
COStBu
OMe
H
OBPS
OPMB
Me
Me
O
O
Me
OBOM
CO2Me
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

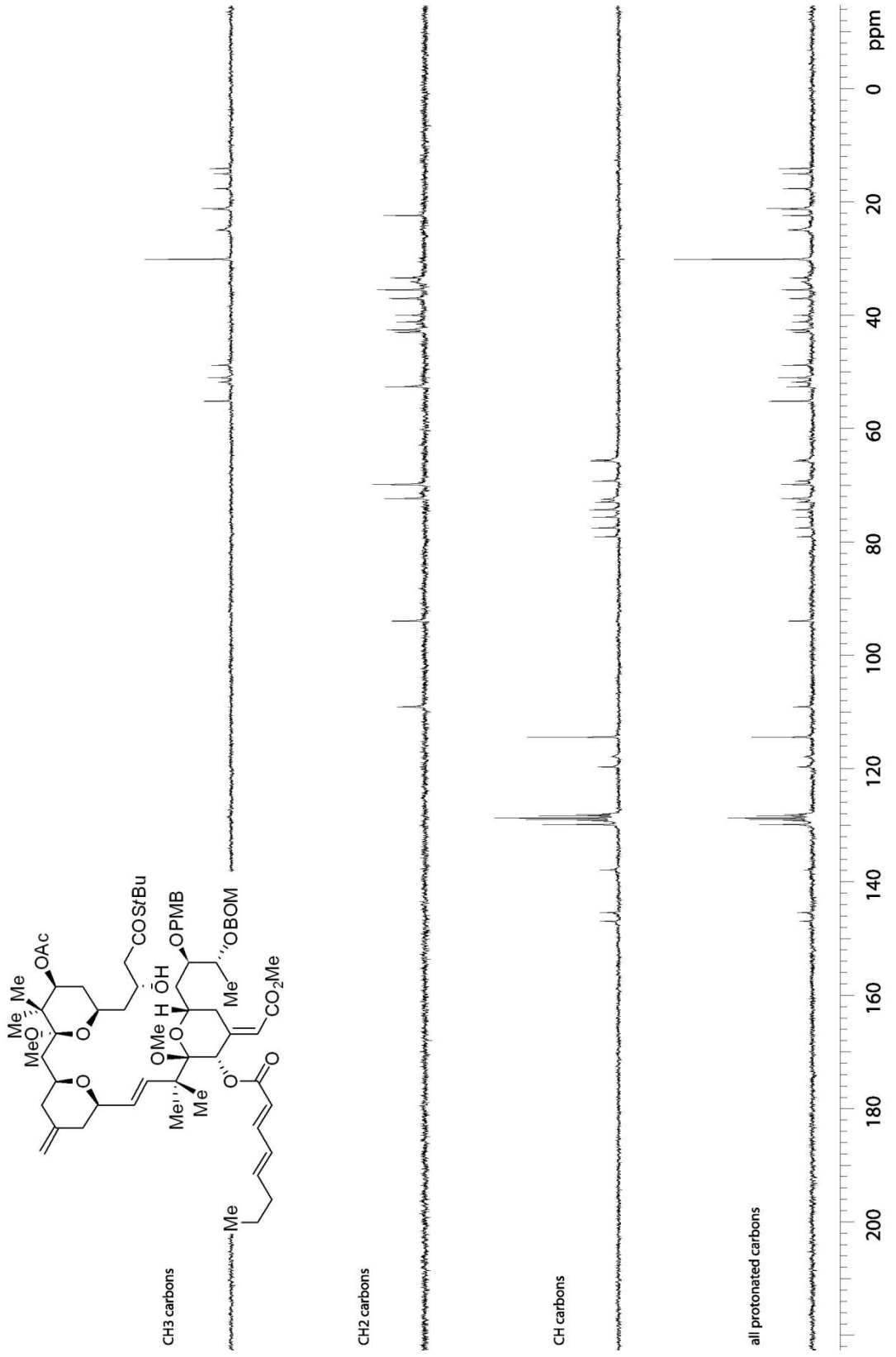

Me
Me
MeO
OAc
O
O
COS*t*Bu
OMe
H
OBPS
OPMB
Me
Me
Me
O
Me
OBOM
O
O
CO_2Me
10
9
8
7
6
5
4
3
2
1
-0
ppm

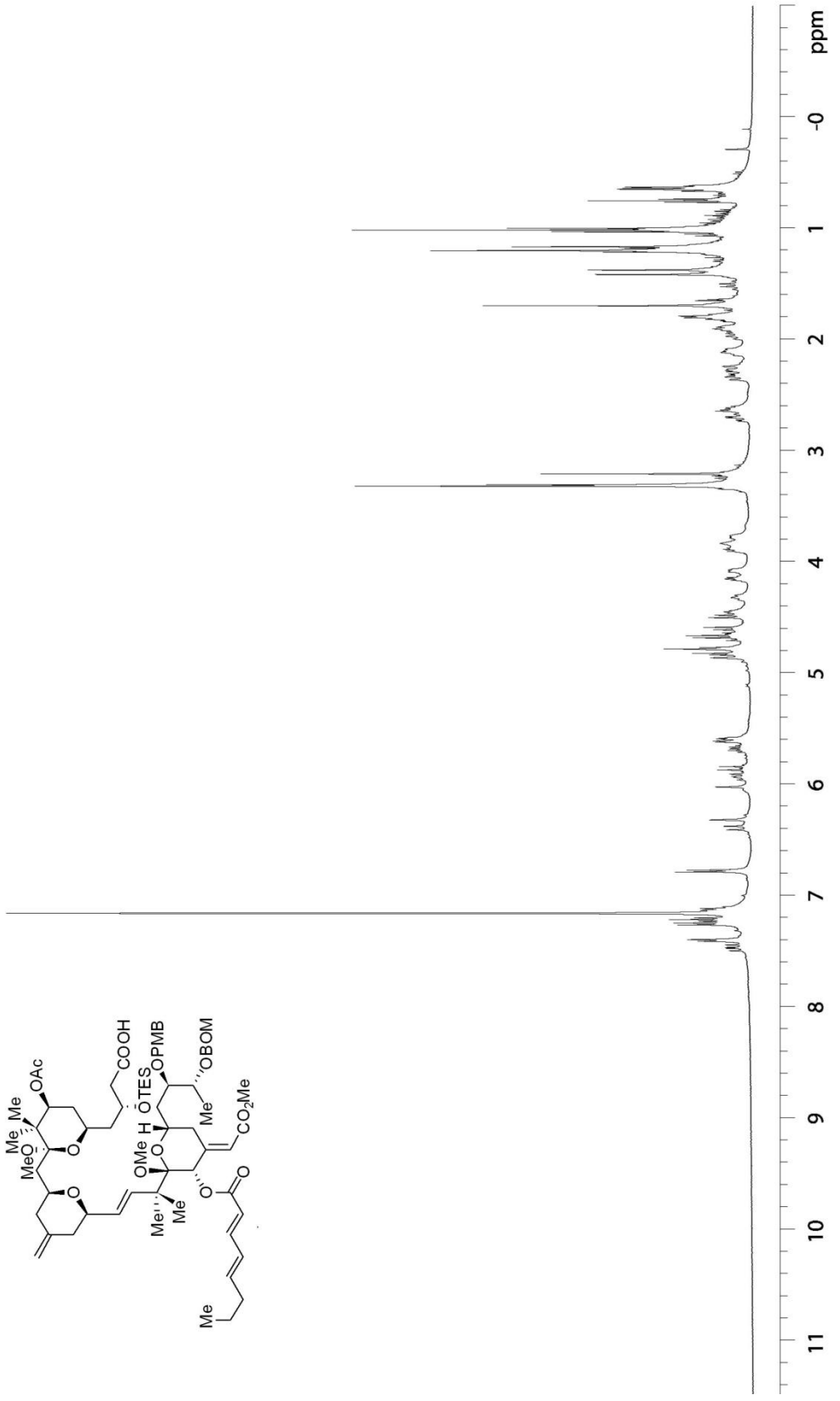

Me
Me
MeO
OAc
COStBu
OMe
H
OBPS
OPMB
Me
Me
Me
OBOM
O
CO2Me
200
180
160
140
120
100
80
60
40
20
ppm

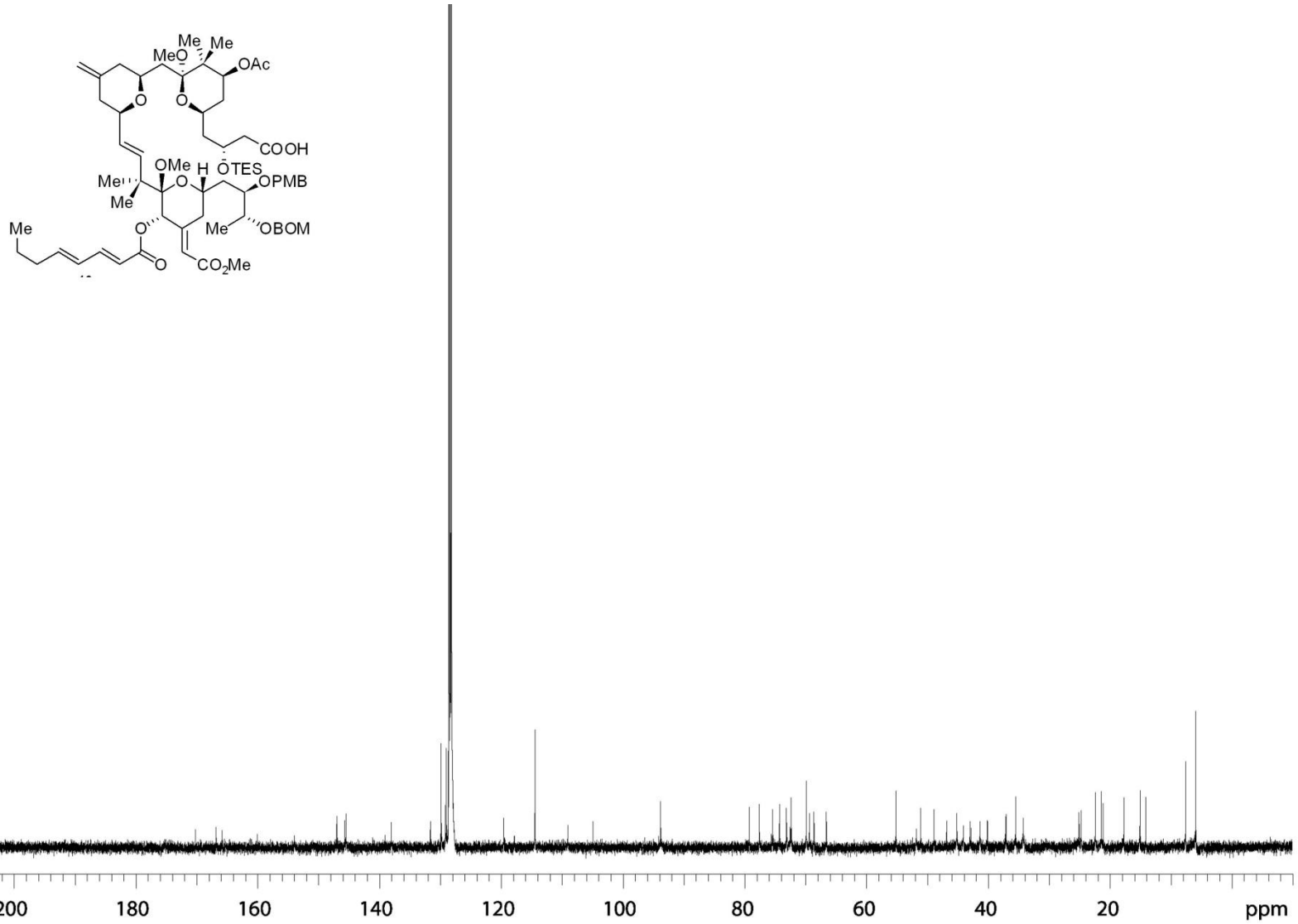

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

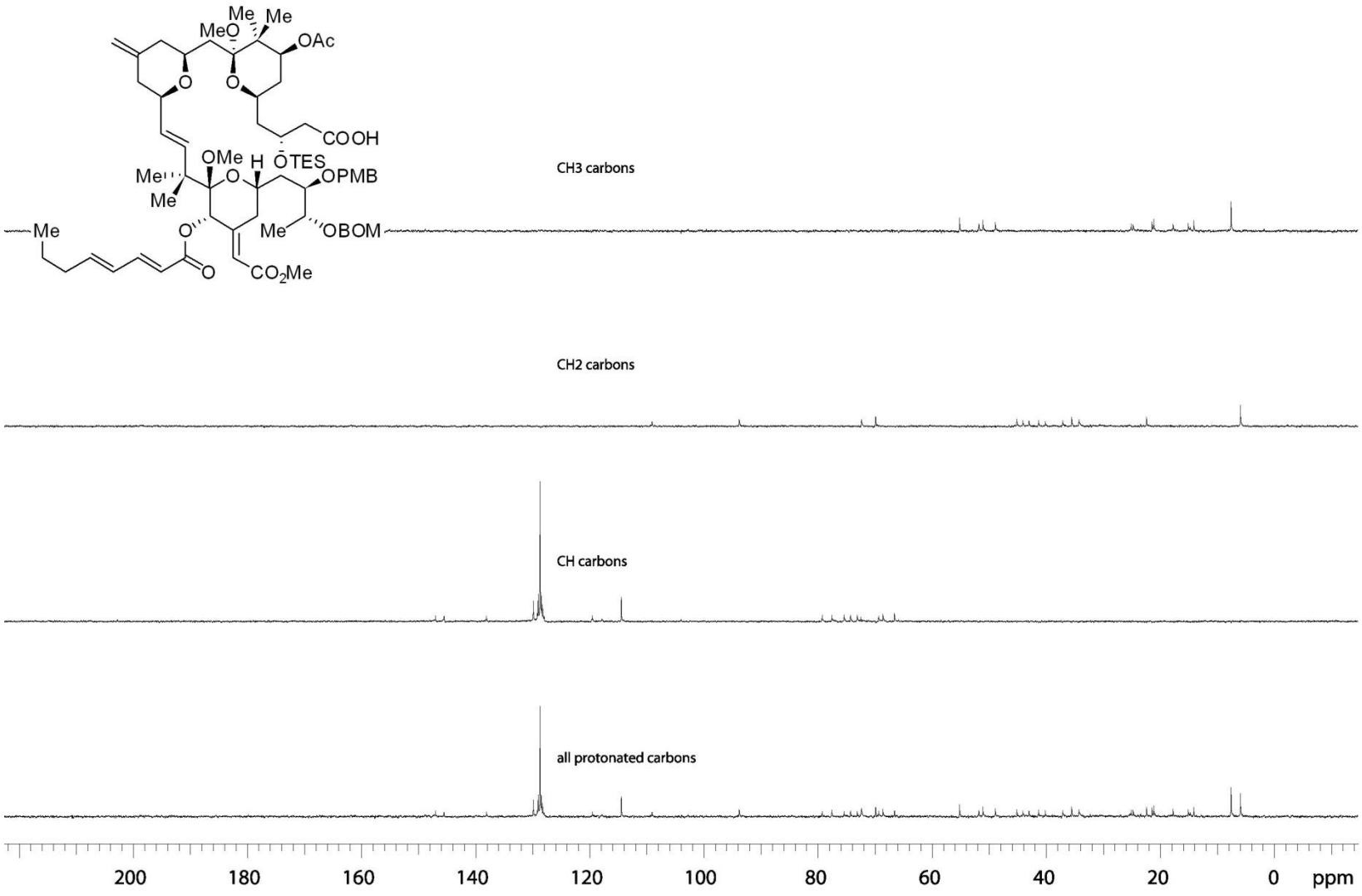

Me
Me
MeO
OAc
O
O
COStBu
OMe
H
OH
OPMB
Me
Me
O
O
Me
OBOM
CO2Me
O
O
Me
ppm
10
9
8
7
6
5
4
3
2
1
-0

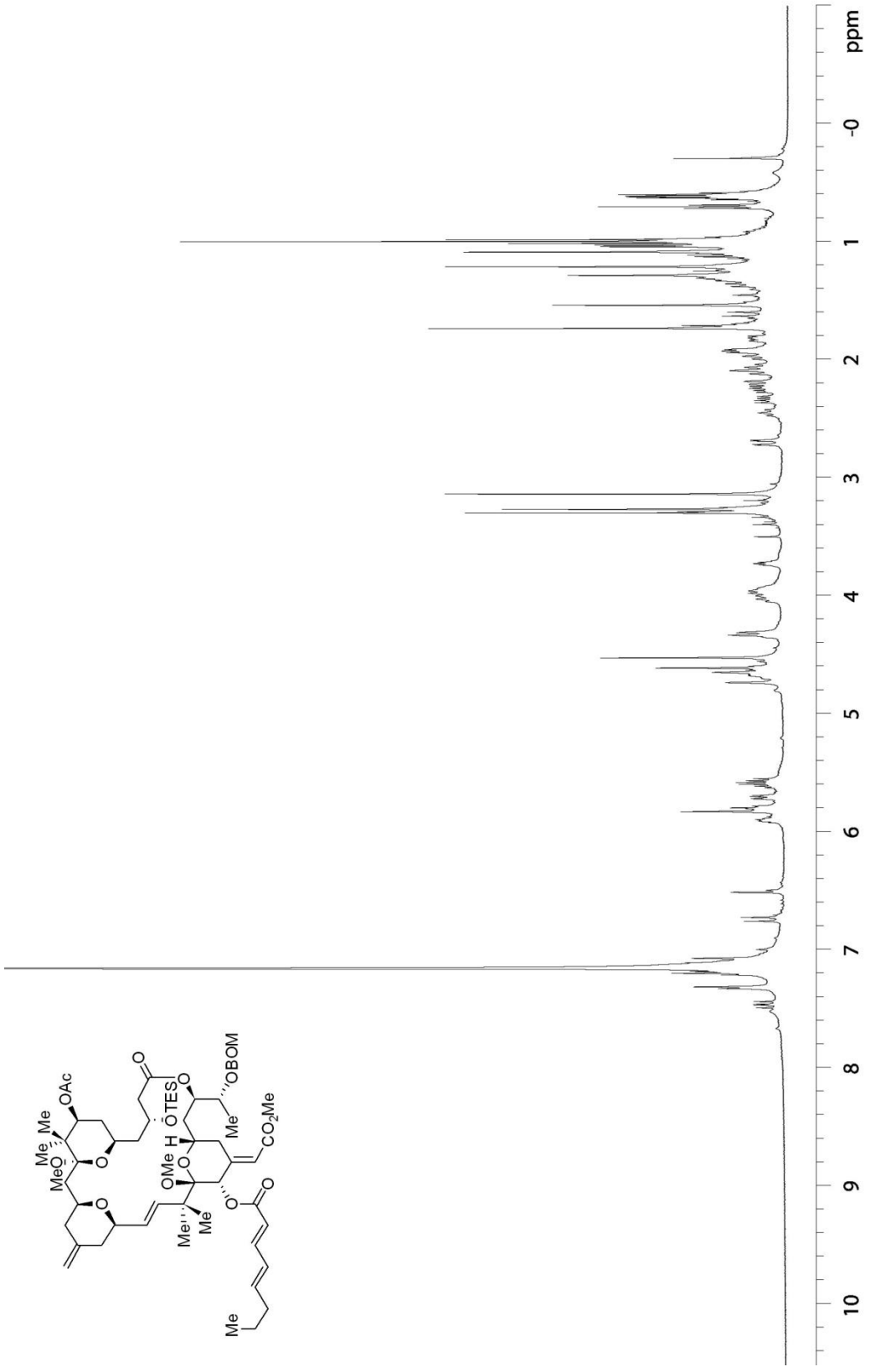

Me
Me
MeO
OAc
O
O
COStBu
OMe
H
OH
Me
Me
OPMB
Me
Me
OBOM
O
O
CO2Me
200
180
160
140
120
100
80
60
40
20
ppm

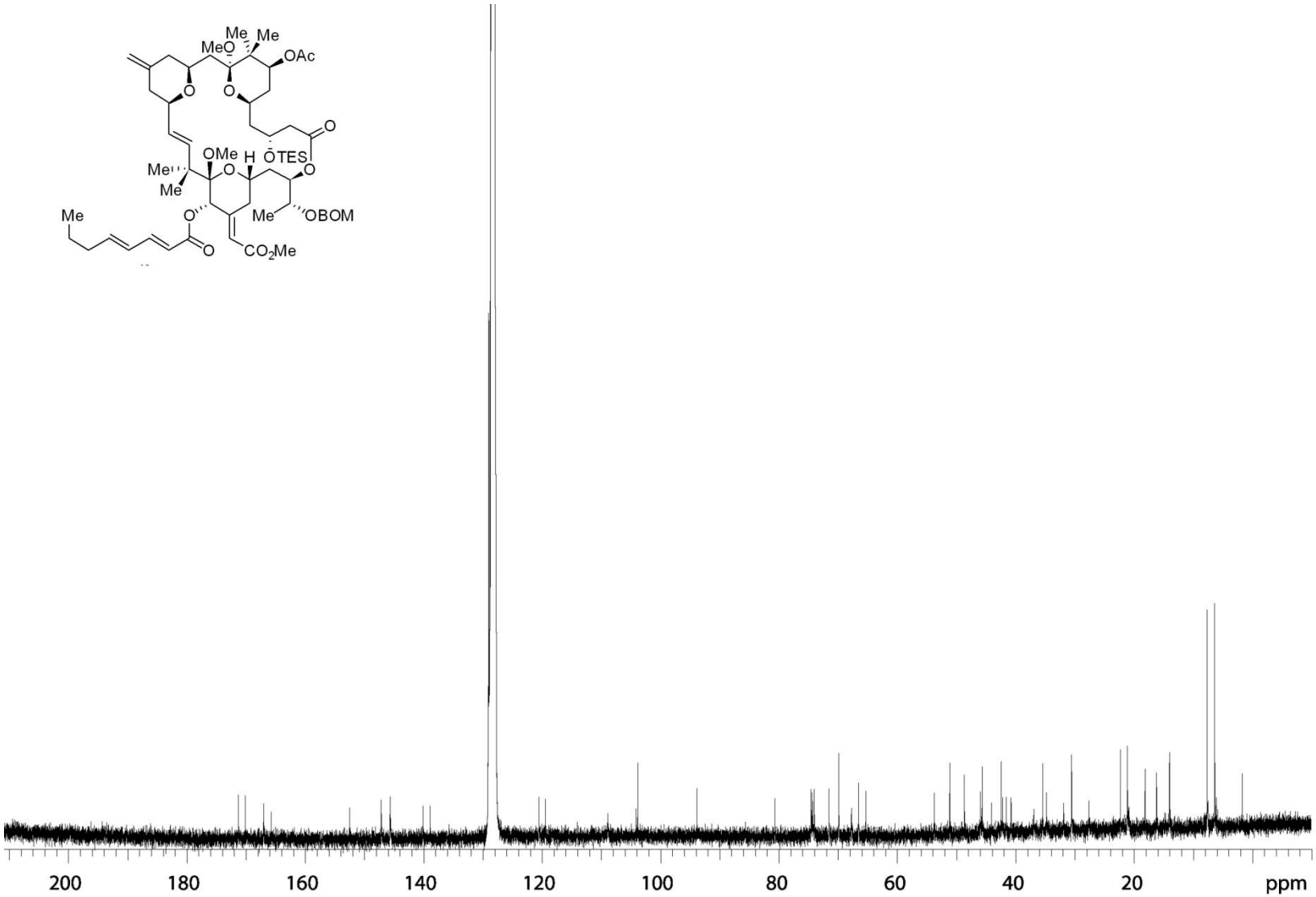

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

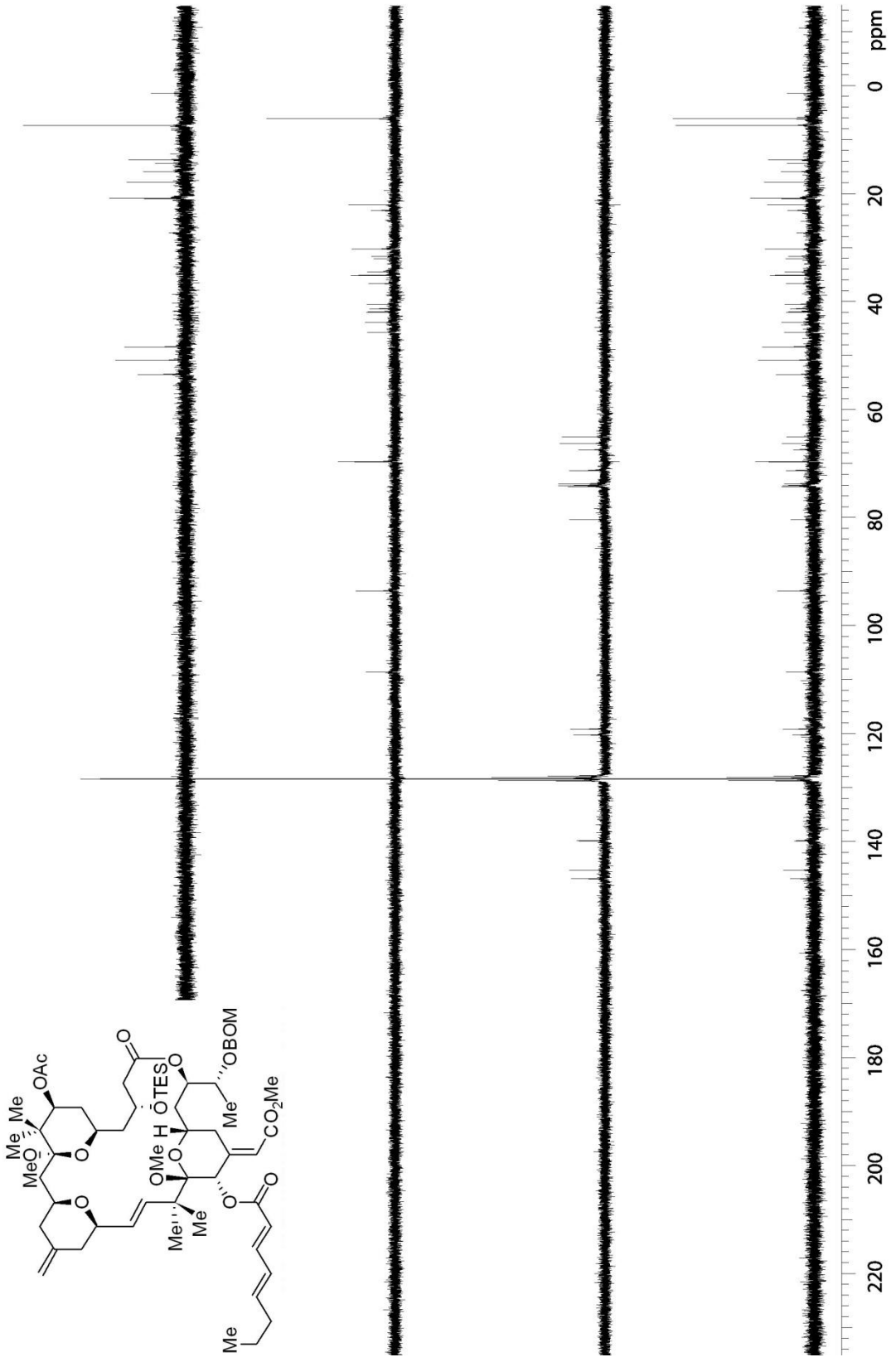

COOH
OAc
OMe
OTES
OPMB
OBOM
CO2Me
Me
MeO
H
O
ppm
11
10
9
8
7
6
5
4
3
2
1
-0

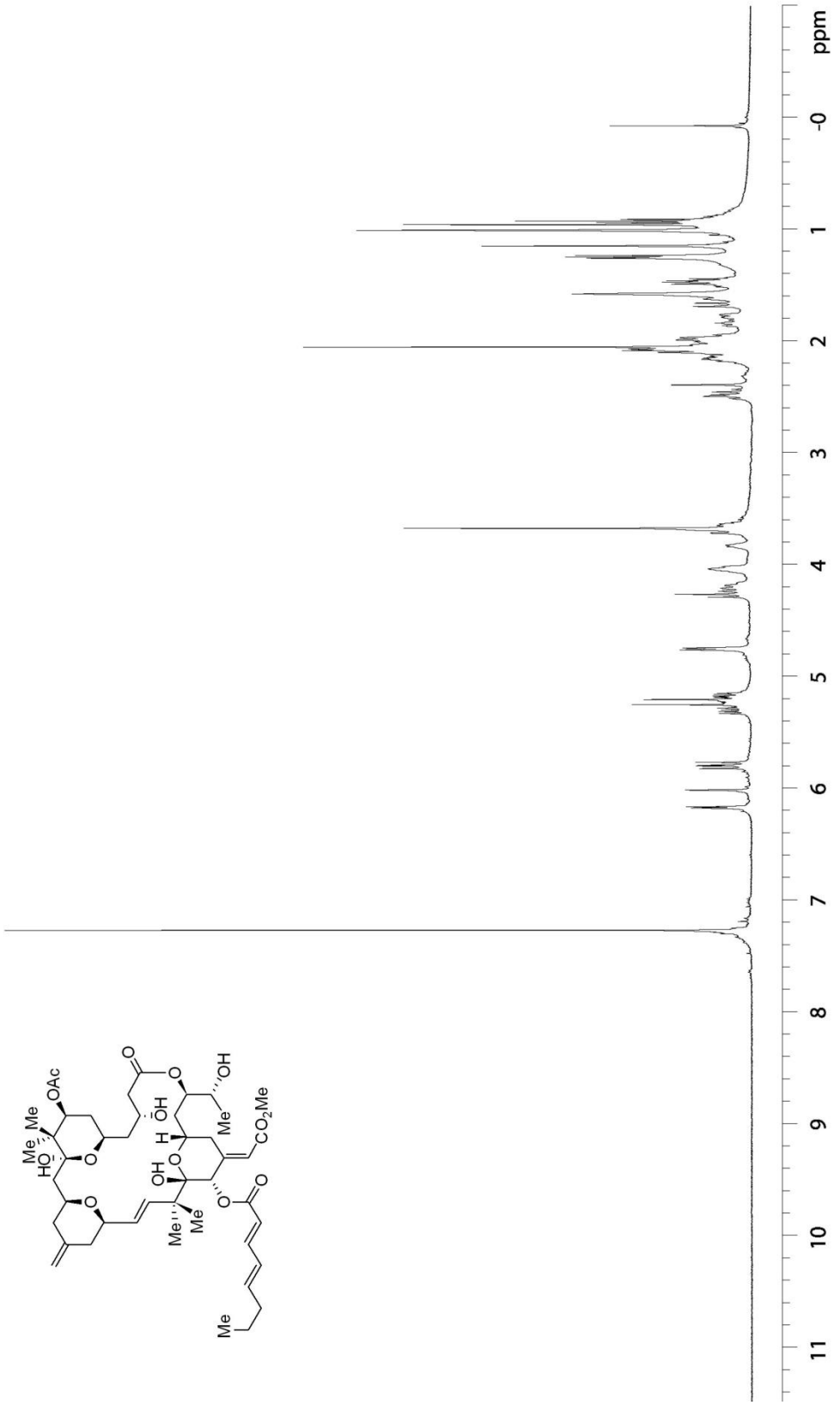

Me
Me
MeO
OAc
O
O
COOH
OMe
H
OTES
Me
Me
OPMB
Me
O
Me
OBOM
Me
O
O
CO2Me
200
180
160
140
120
100
80
60
40
20
ppm

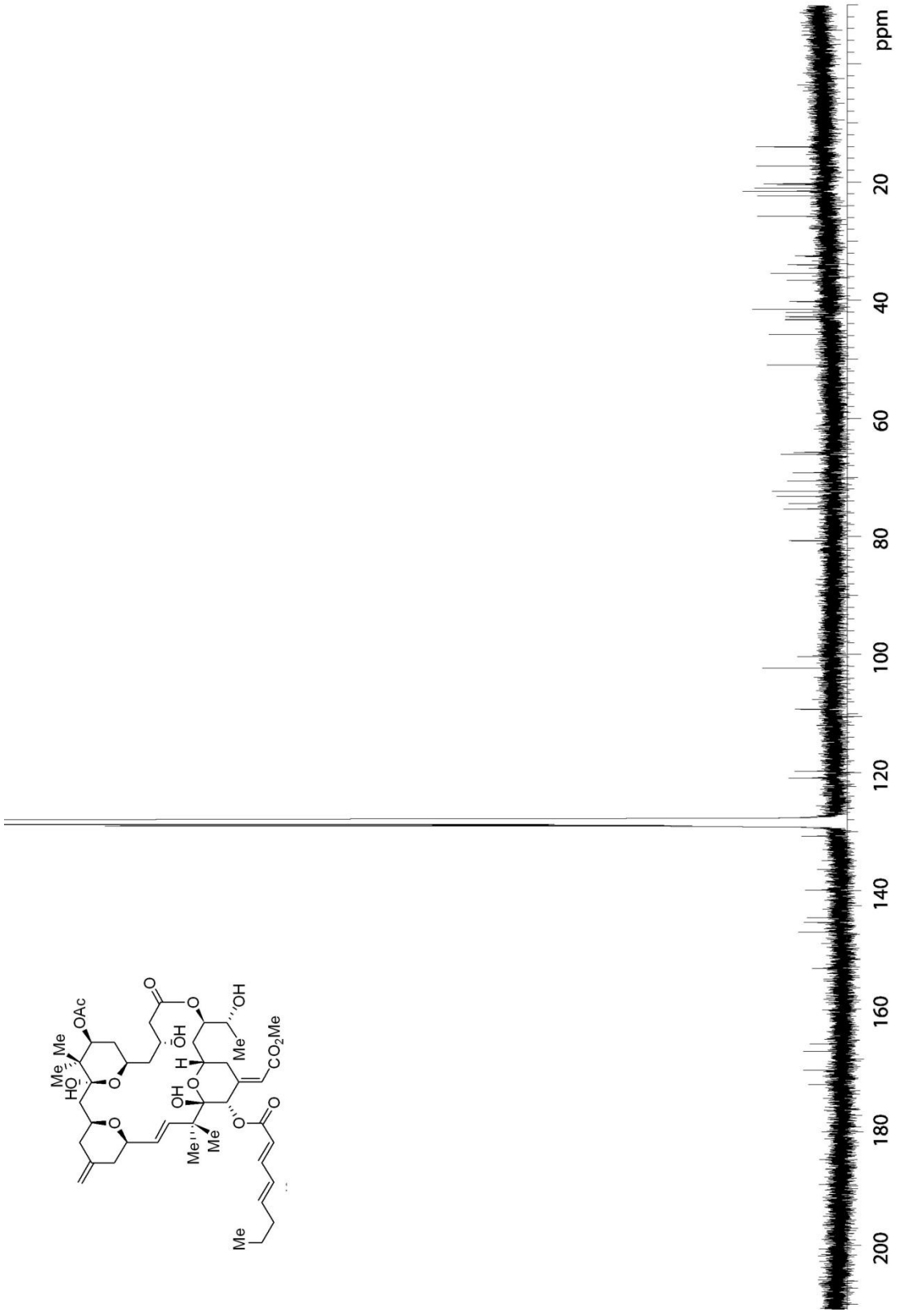

Me
Me
MeO
OAc
O
O
COOH
OMe
H
OTES
OPMB
Me
Me
Me
O
Me
OBOM
O
O
CO2Me
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

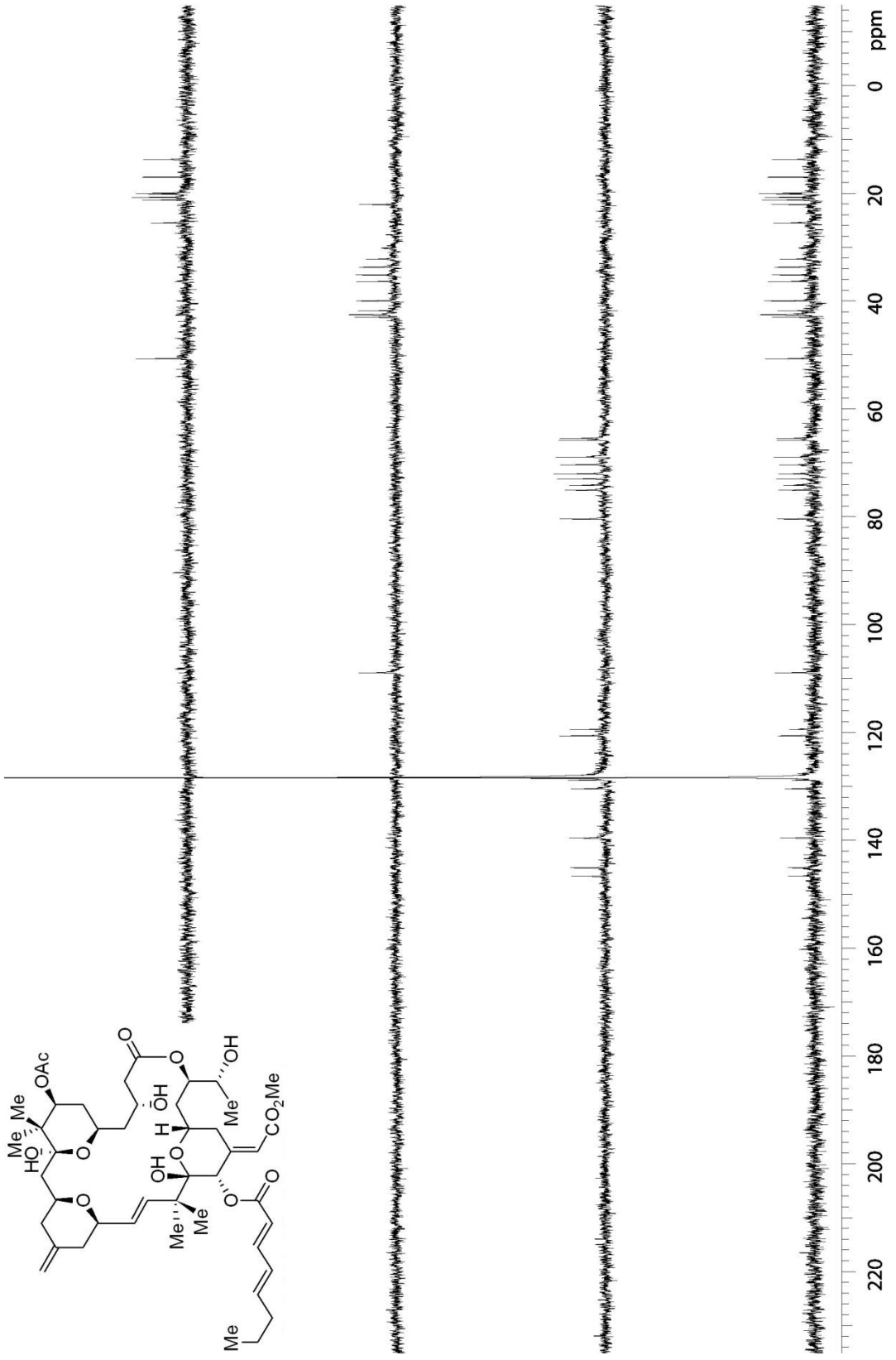

OAc
Me
Me
MeO
OMe
H
OTES
Me
Me
Me
OBOM
CO2Me
Me
O
ppm
10
9
8
7
6
5
4
3
2
1
-0

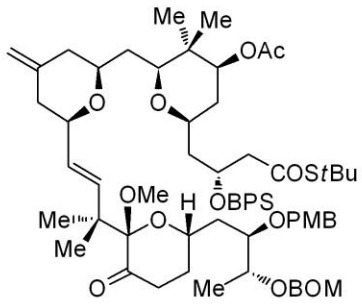

Me
Me
MeO
OAc
O
O
O
OMe
H
OTES
O
Me
Me
Me
OBOM
O
O
CO2Me
200
180
160
140
120
100
80
60
40
20
ppm


OAc
Me
Me
MeO
OMe
H
OTES
OBOM
Me
CO2Me
Me
Me
Me
O
O
O

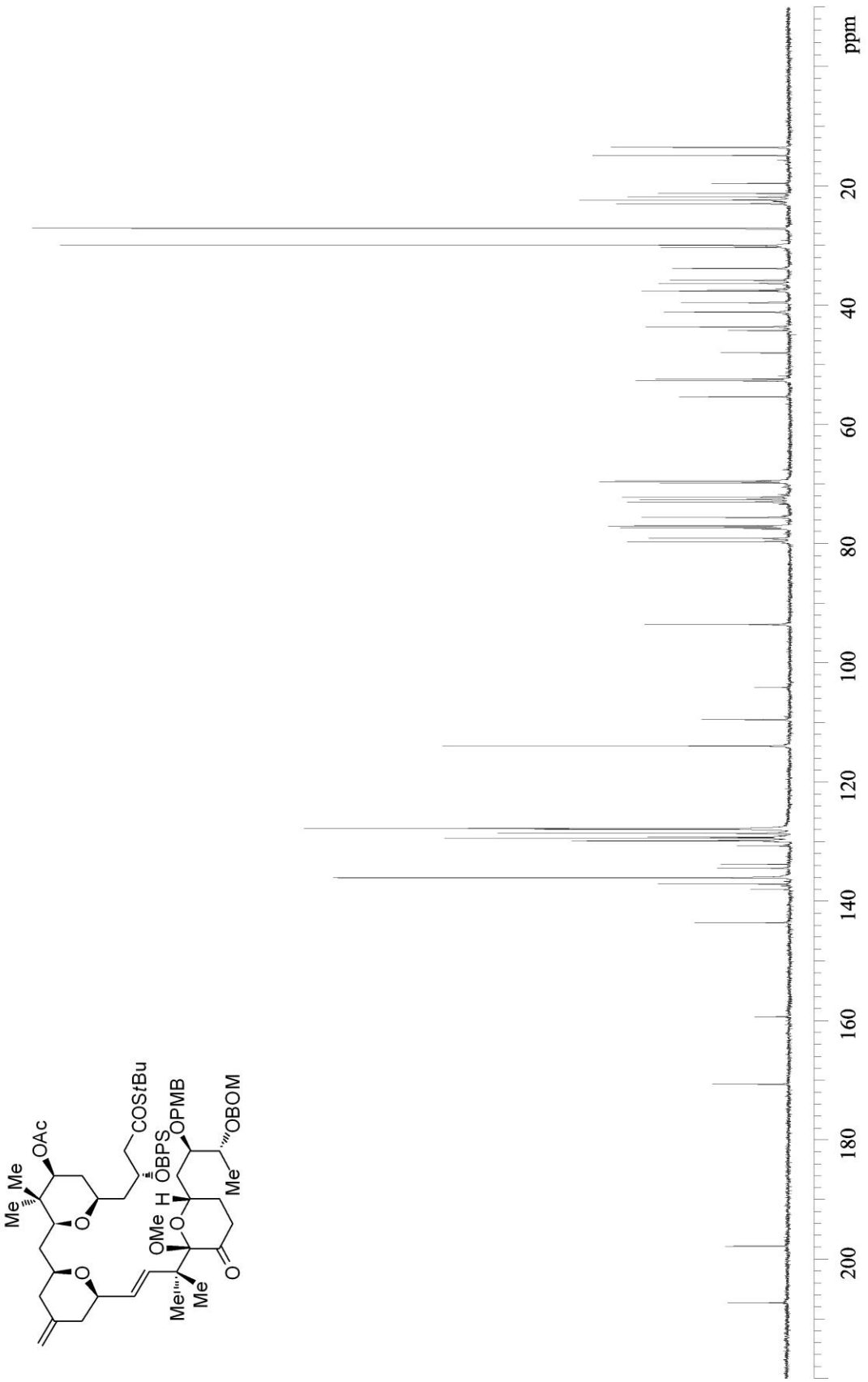

ppm
-0
1
2
3
4
5
6
7
8
9
10
11
OAc
Me
Me
HO
OH
H
OH
Me
OH
CO2Me
Me
Me
Me

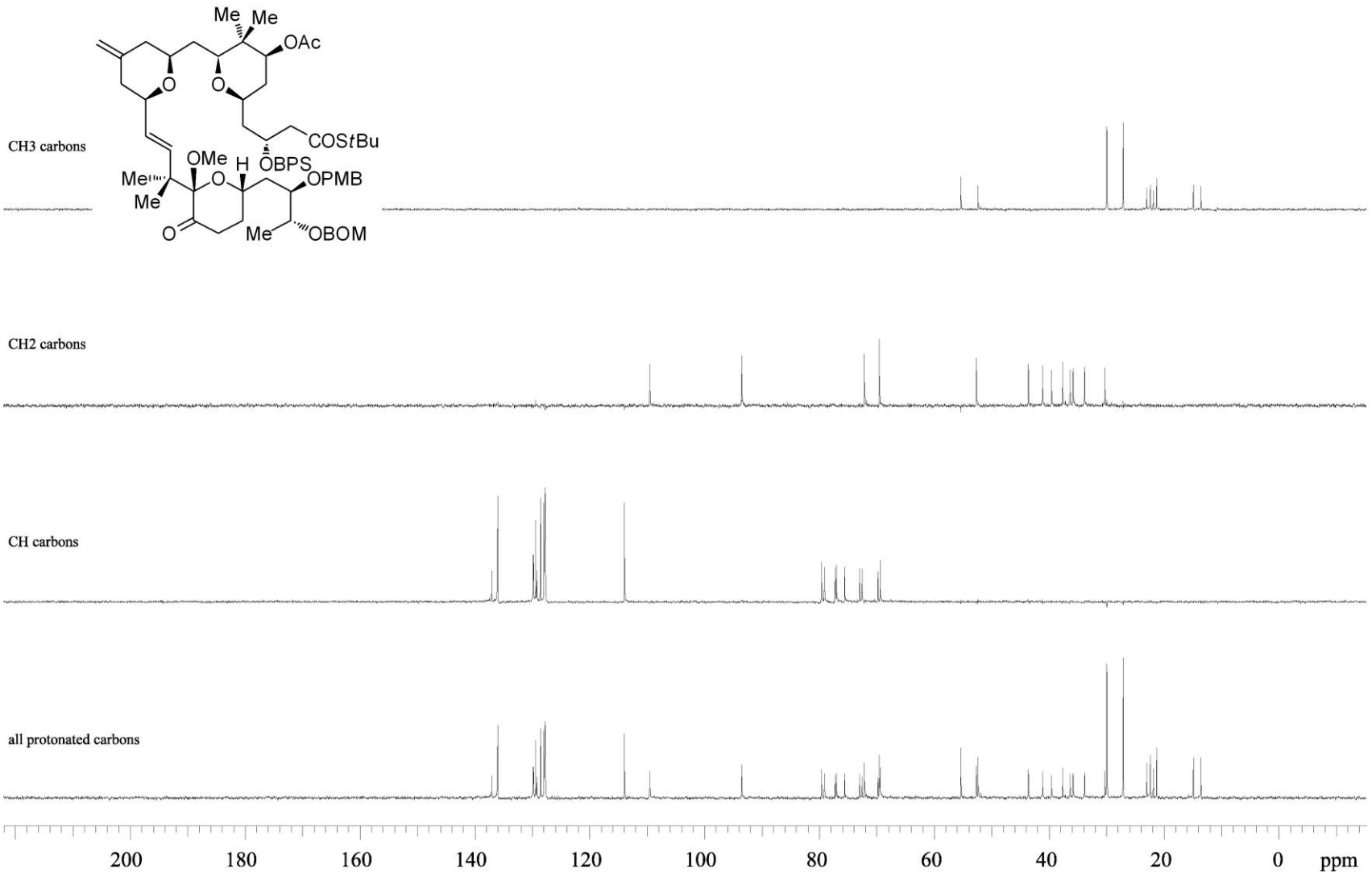

ppm
20
40
60
80
100
120
140
160
180
200
OAc
Me
Me
HO
OH
H
OH
Me
Me
Me
OH
CO2Me
O
O
O

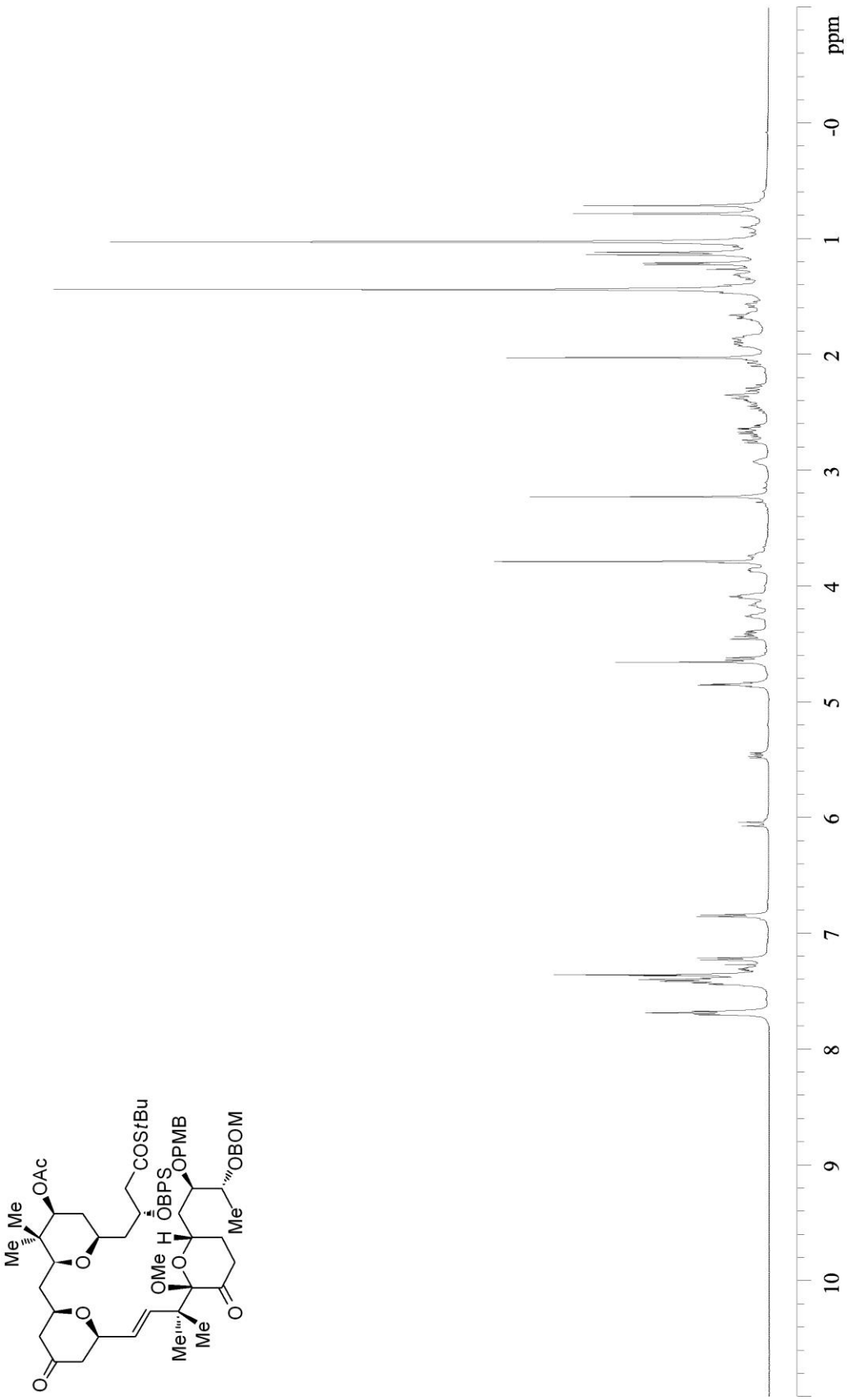

Me
Me
HO
OAc
O
O
OH
H
OH
O
Me
Me
O
O
Me
OH
CO2Me
Me
220
200
180
160
140
120
100
80
60
40
20
0
ppm

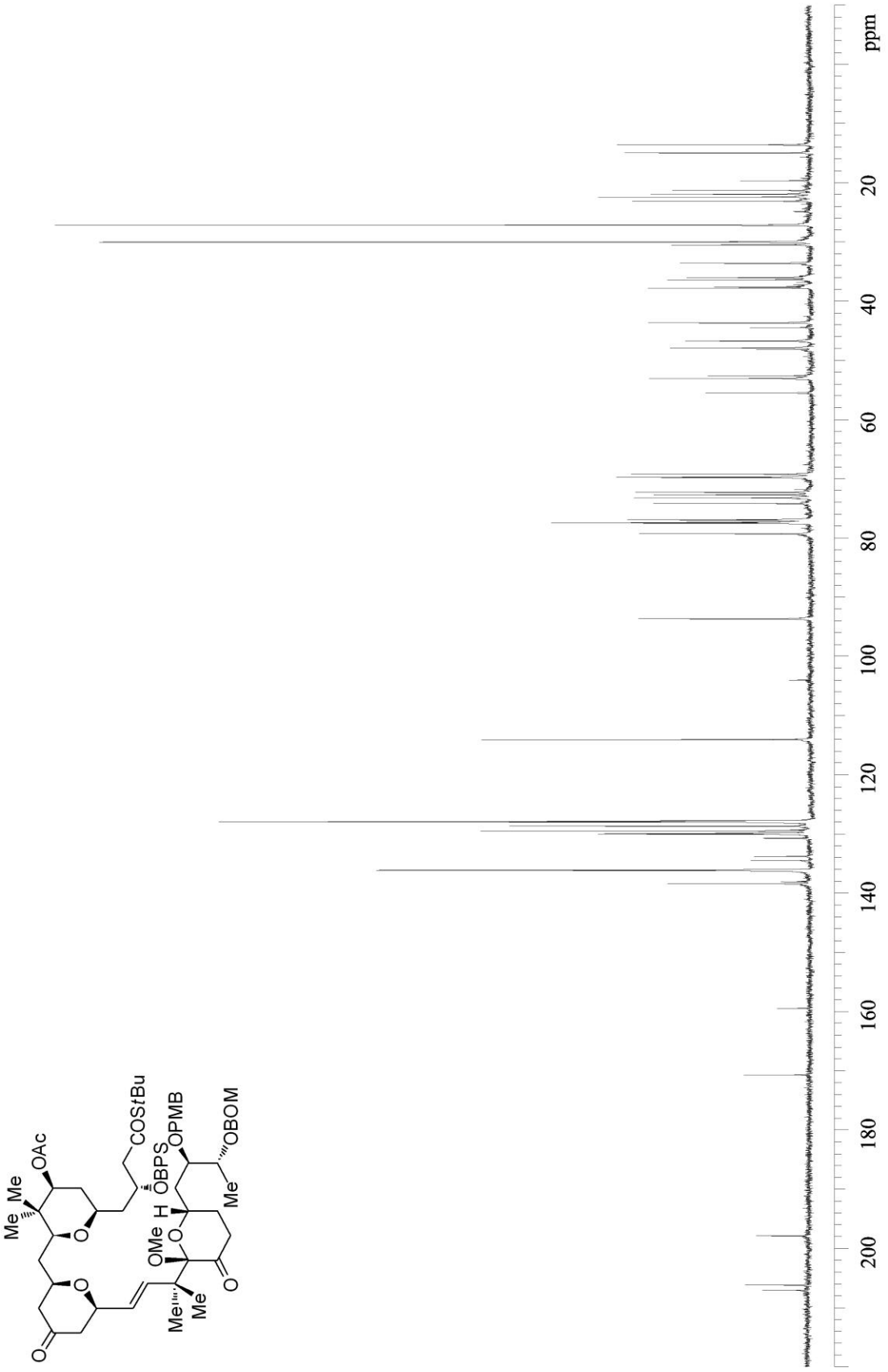

Me
Me
OAc
O
O
COS*t*Bu
OMe
H
OBPS
Me
O
OPMB
Me
O
Me
OBOM

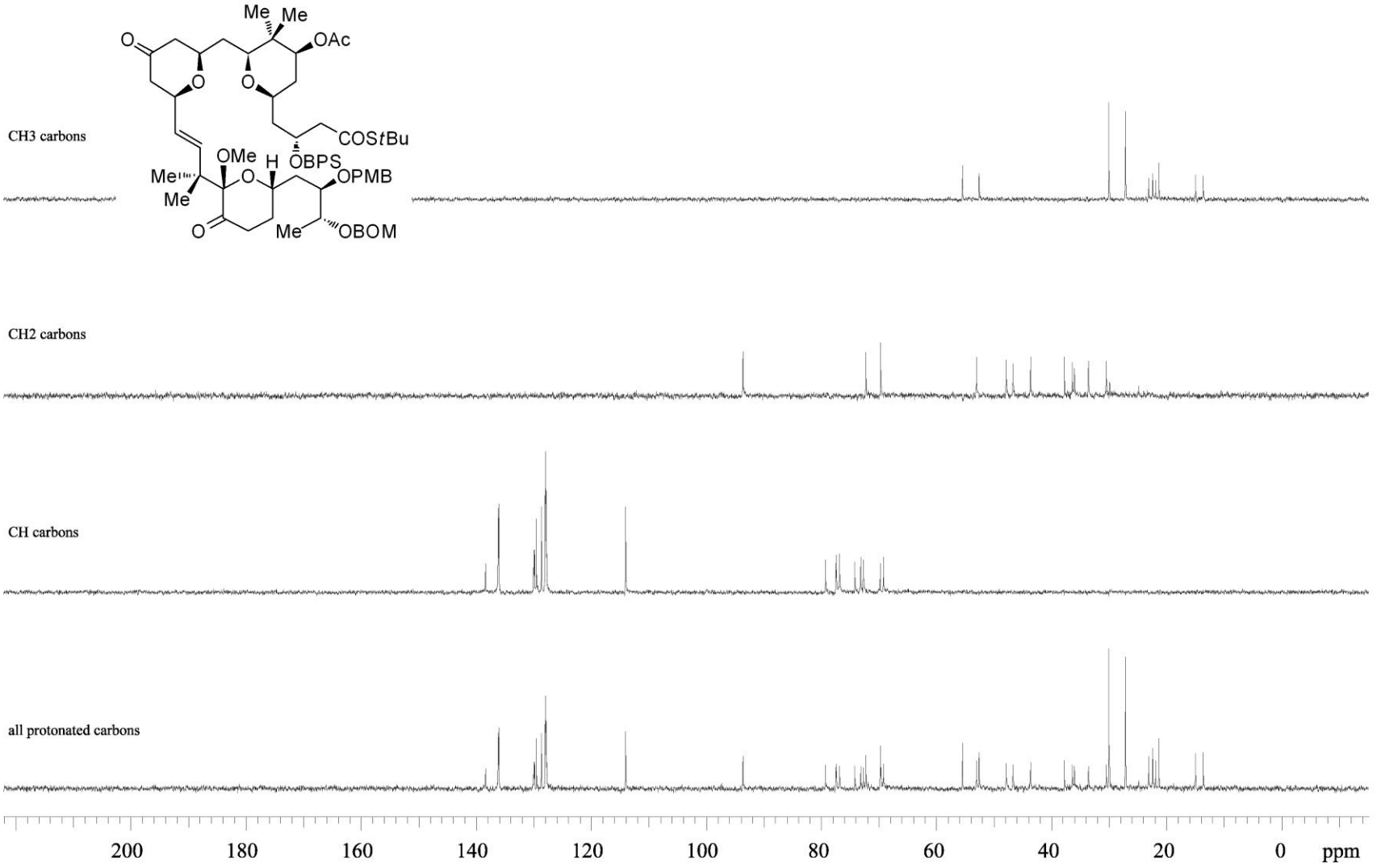
10
9
8
7
6
5
4
3
2
1
-0
ppm

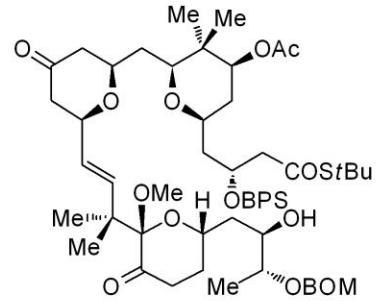

Me
Me
OAc
O
O
OMe
H
O
O
Me
Me
OBPS
COStBu
OPMB
Me
OBOM
200
180
160
140
120
100
80
60
40
20
ppm


Me
Me
OAc
O
O
COS*t*Bu
OMe
H
OBPS
Me
O
OPMB
Me
O
Me
OBOM
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

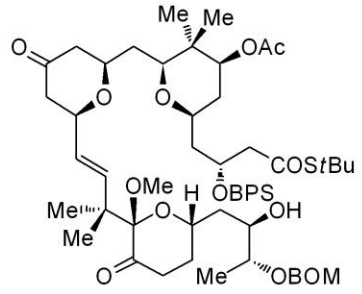

Me
Me
OAc
O
O
O
COStBu
H
OBPS
OMe
O
OPMB
Me
Me
Me
OBOM
O
10
9
8
7
6
5
4
3
2
1
-0
ppm


Me
Me
OAc
O
O
O
COStBu
OBPS
H
OMe
O
OPMB
OBOM
Me
Me
Me
Me
O
O
ppm
200
180
160
140
120
100
80
60
40
20

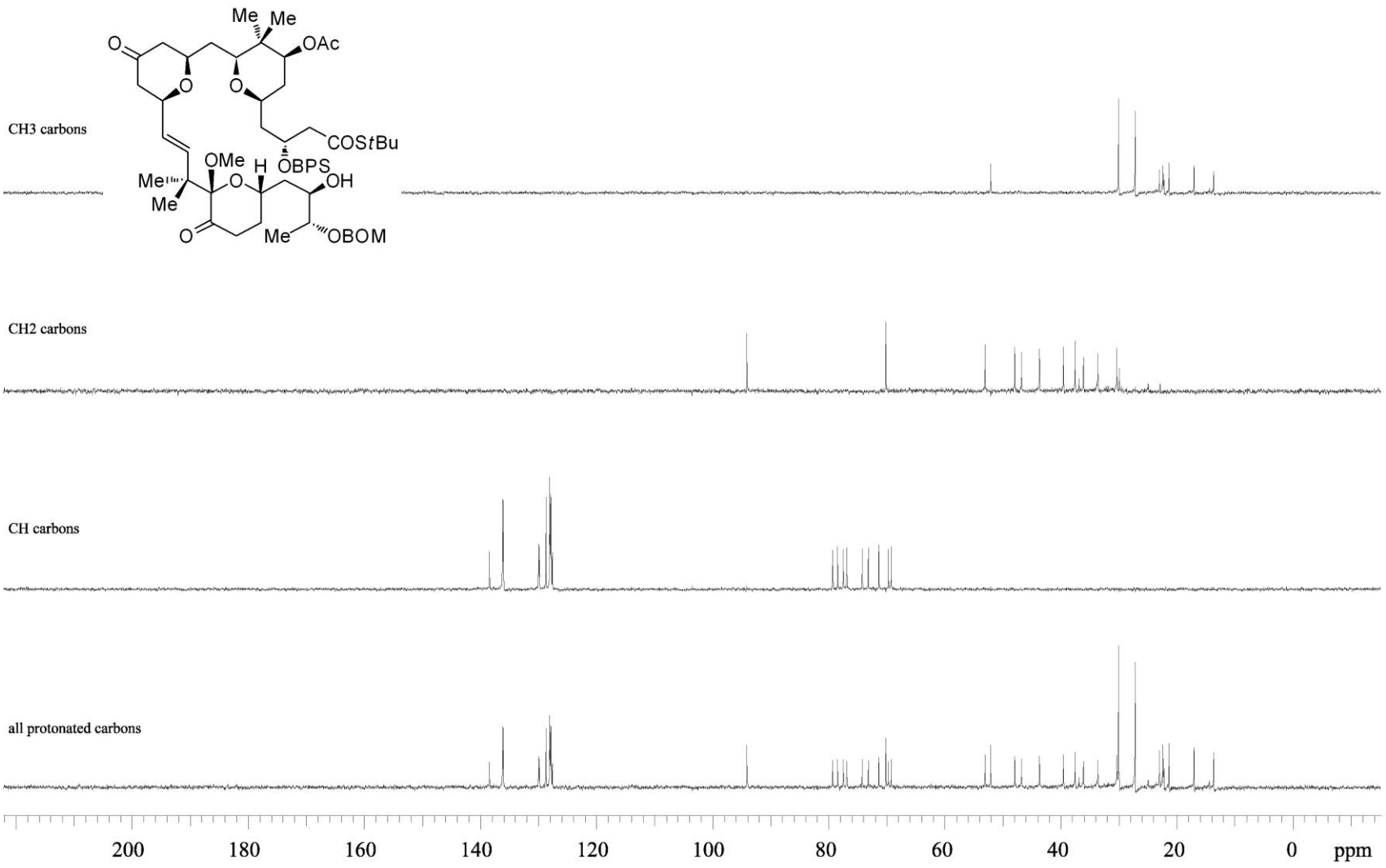

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
Me
Me
OAc
O
O
O
COStBu
OMe
H
OBPS
OPMB
Me
Me
O
Me
OBOM
200
180
160
140
120
100
80
60
40
20
0
ppm

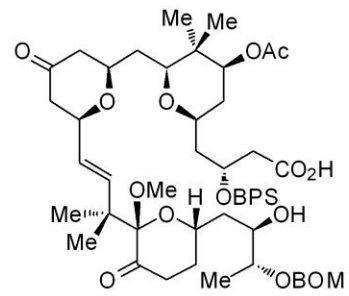

Me
Me
OAc
O
O
O
COS*t*Bu
OMe
H
OBPS
Me
O
OH
Me
O
Me
OBOM

10
9
8
7
6
5
4
3
2
1
-0
ppm

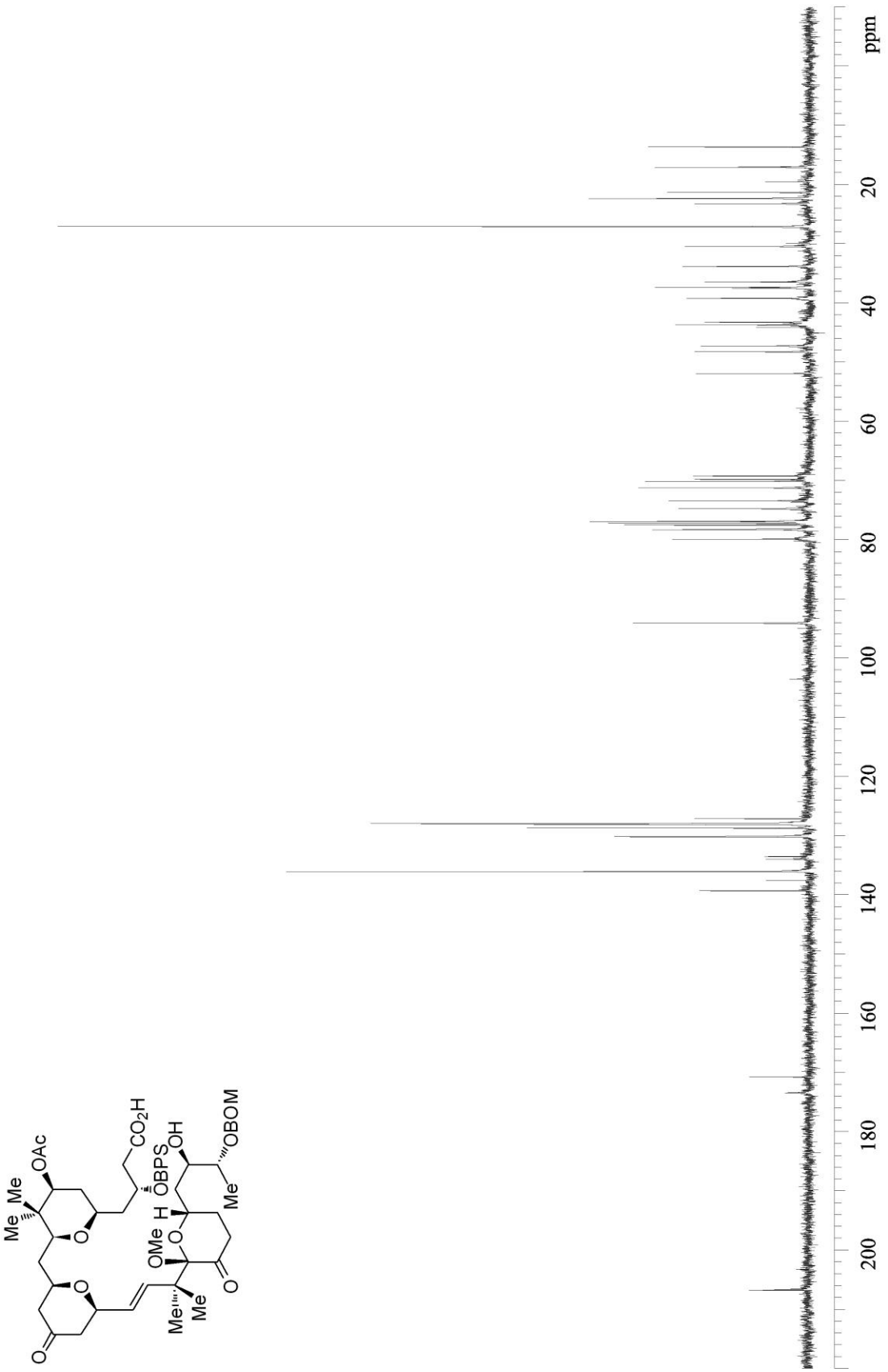

Me
Me
OAc
O
O
O
COS*t*Bu
OMe
H
OBPS
Me
Me
O
OH
O
Me
OBOM

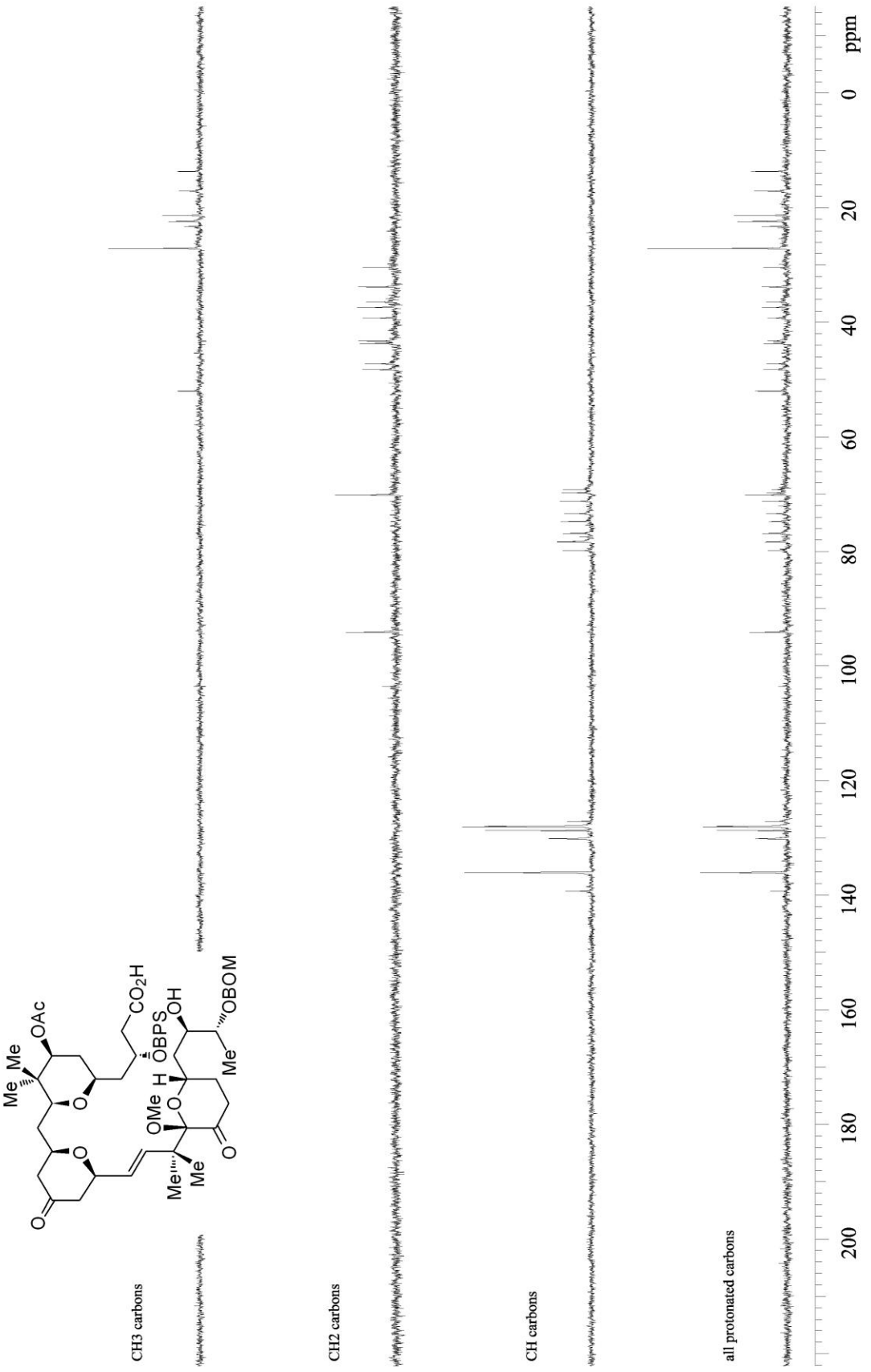
200
180
160
140
120
100
80
60
40
20
ppm

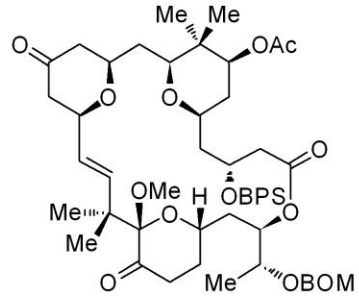

Me
Me
OAc
O
O
O
COStBu
OMe
H
OBPS
OH
Me
Me
O
O
Me
OBOM
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm


Me
Me
OAc
O
O
O
CO2H
OMe
H
OBPS
Me
Me
O
OH
Me
O
Me
OBOM

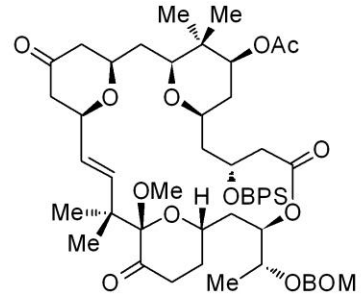
10
9
8
7
6
5
4
3
2
1
-0
ppm


Me
Me
OAc
O
O
O
CO2H
OBPS
H
OMe
O
OH
Me
OBOM
Me
Me
O
ppm
200
180
160
140
120
100
80
60
40
20

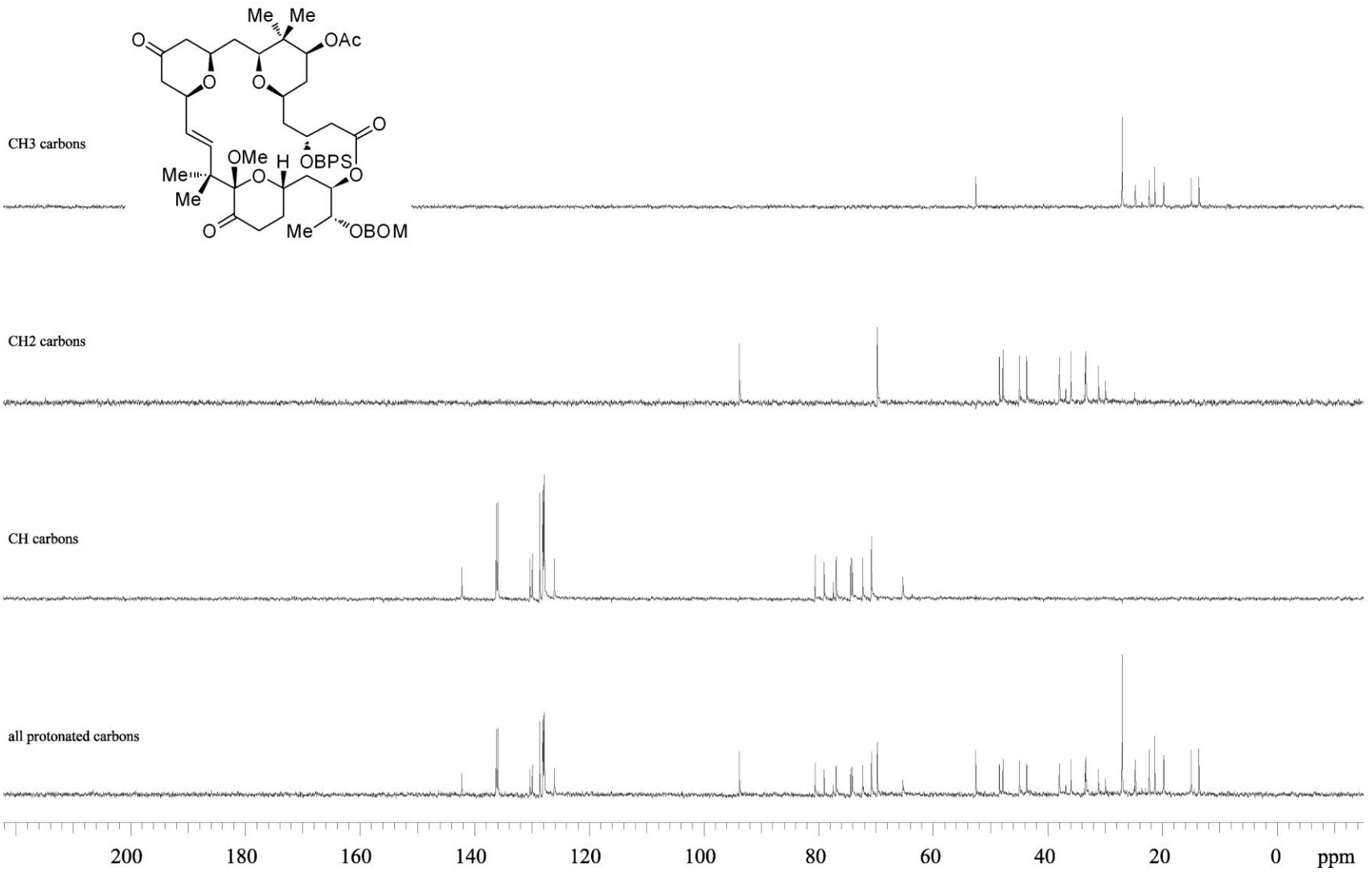

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
Me
Me
OAc
O
O
O
CO2H
OMe
H
OBPS
OH
O
Me
Me
Me
OBOM
O
200
180
160
140
120
100
80
60
40
20
0
ppm

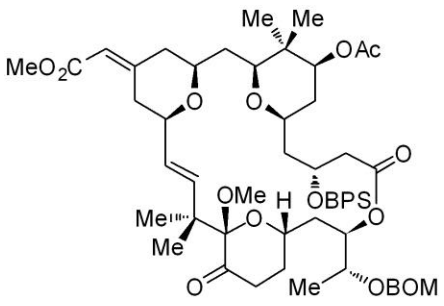

Me
Me
OAc
O
O
O
O
OMe
H
OBPS
O
Me
Me
O
O
Me
OBOM

10
9
8
7
6
5
4
3
2
1
-0
ppm

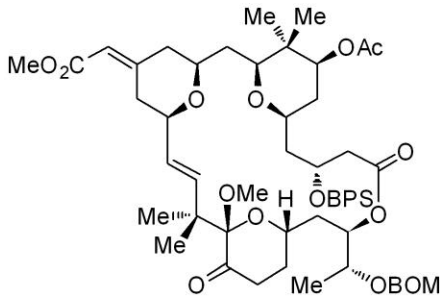

Me
Me
OAc
O
O
O
O
OMe
H
OBPS
Me
O
O
Me
Me
O
Me
OBOM

200
180
160
140
120
100
80
60
40
20
ppm

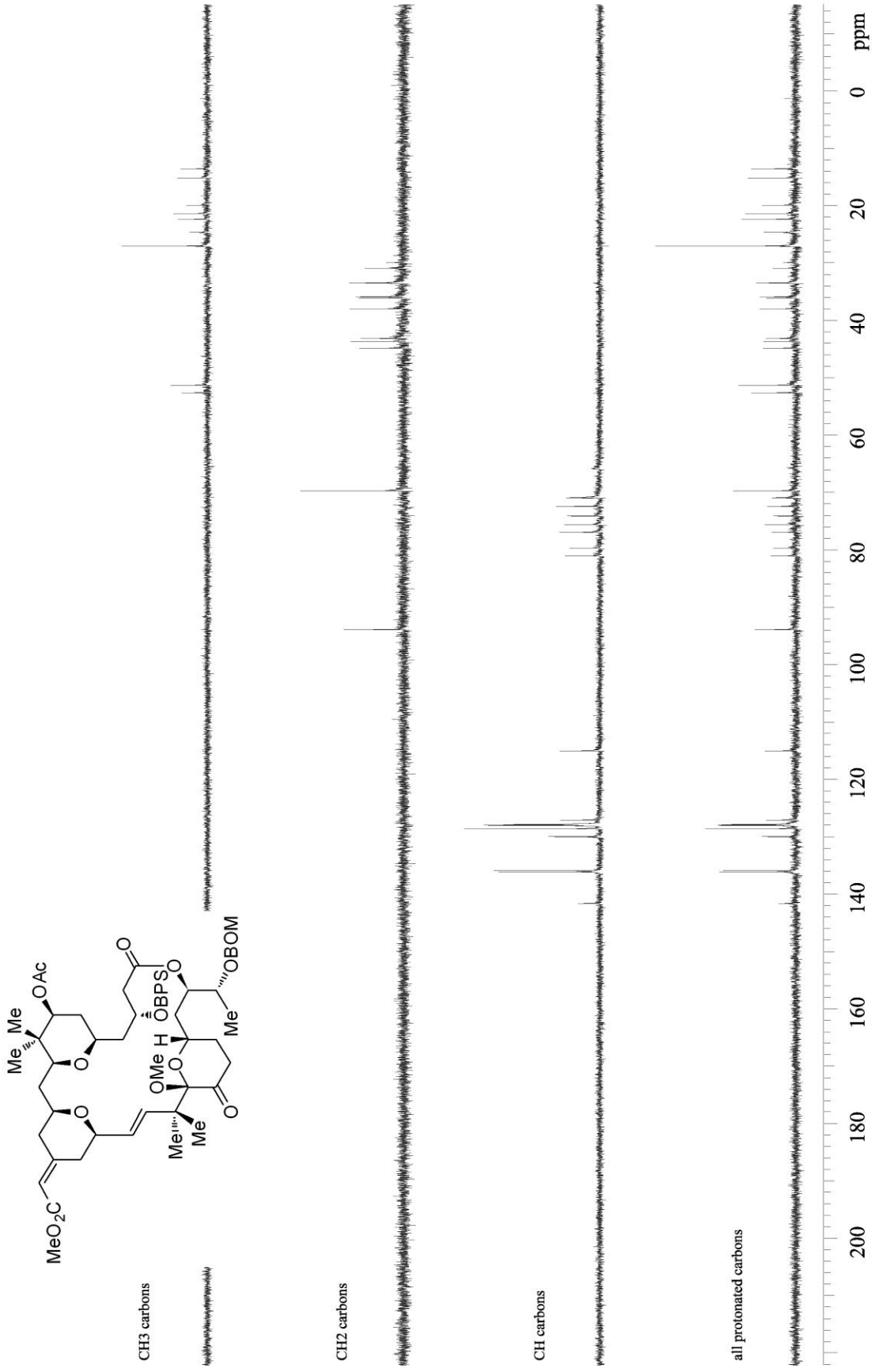

Me
Me
OAc
O
O
O
O
OMe
H
OBPS
Me
O
O
Me
O
Me
OBOM
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

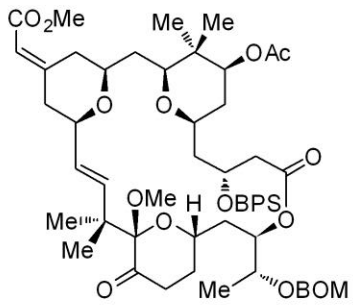

Me
Me
MeO2C
OAc
O
O
O
OMe
H
OBPS
O
Me
Me
O
O
Me
OBOM

10
9
8
7
6
5
4
3
2
1
-0
ppm

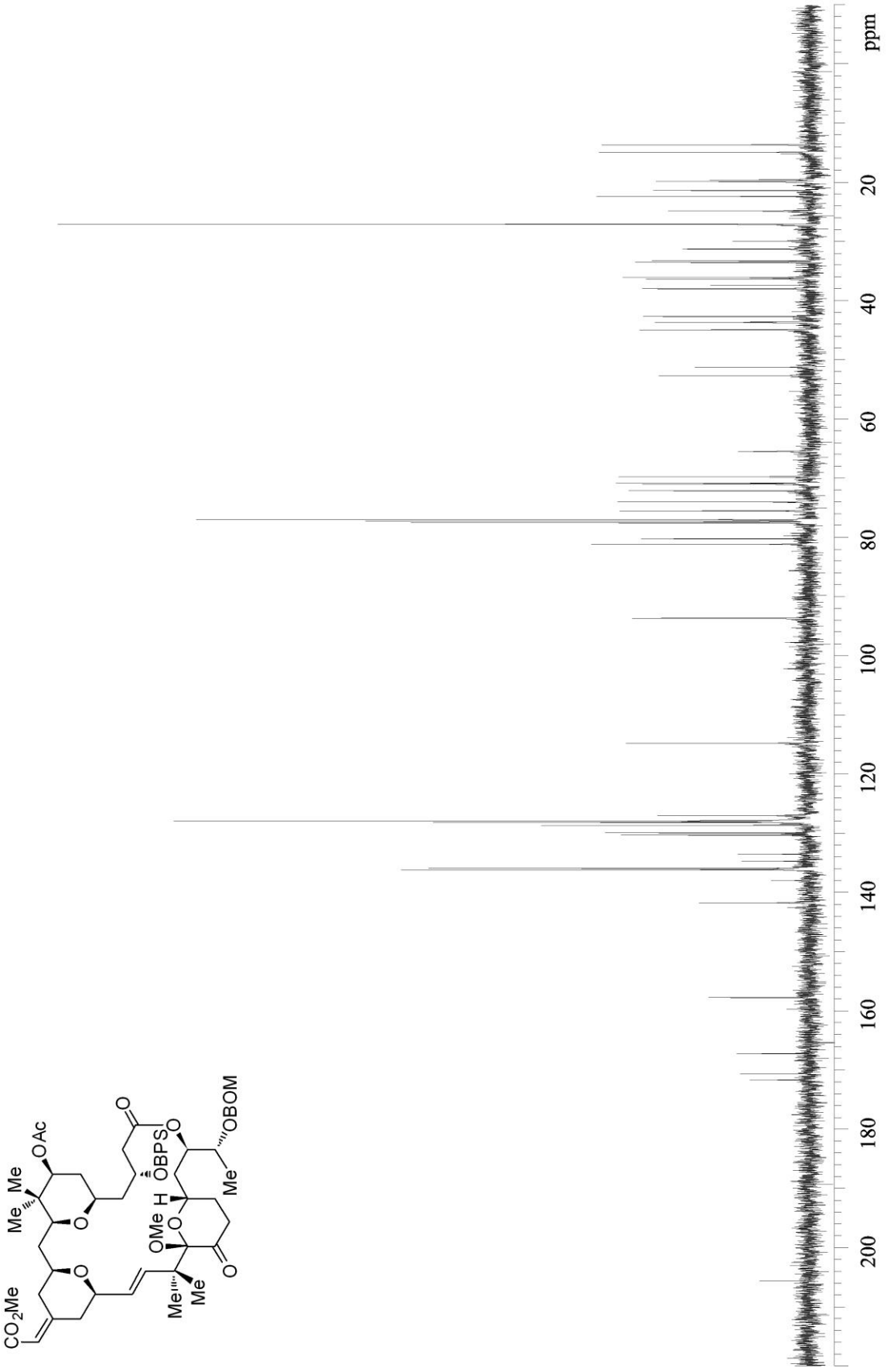

Me
Me
OAc
MeO2C
O
O
O
OMe
H
OBPS
O
Me
Me
O
O
Me
OBOM

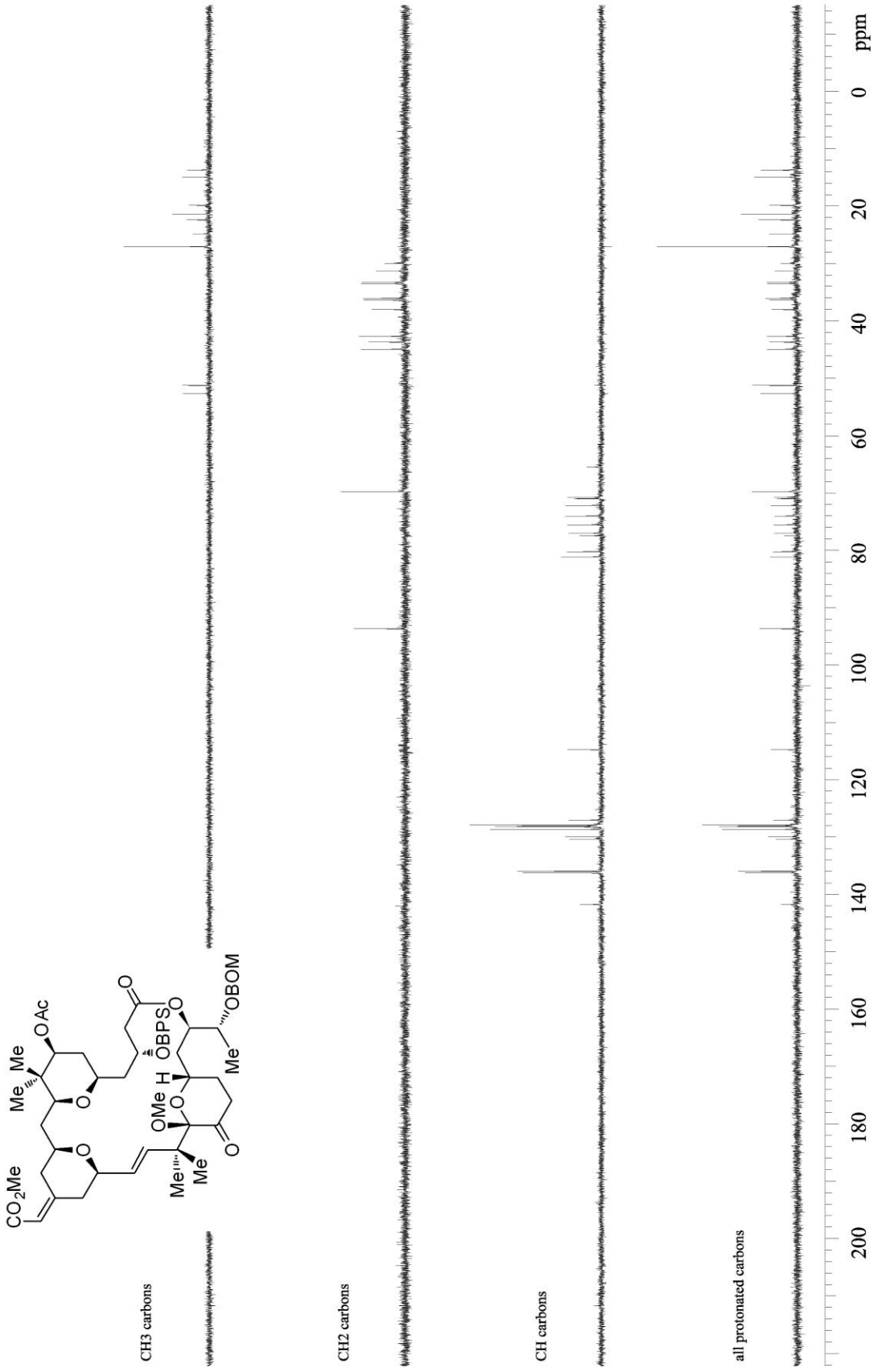
200
180
160
140
120
100
80
60
40
20
ppm

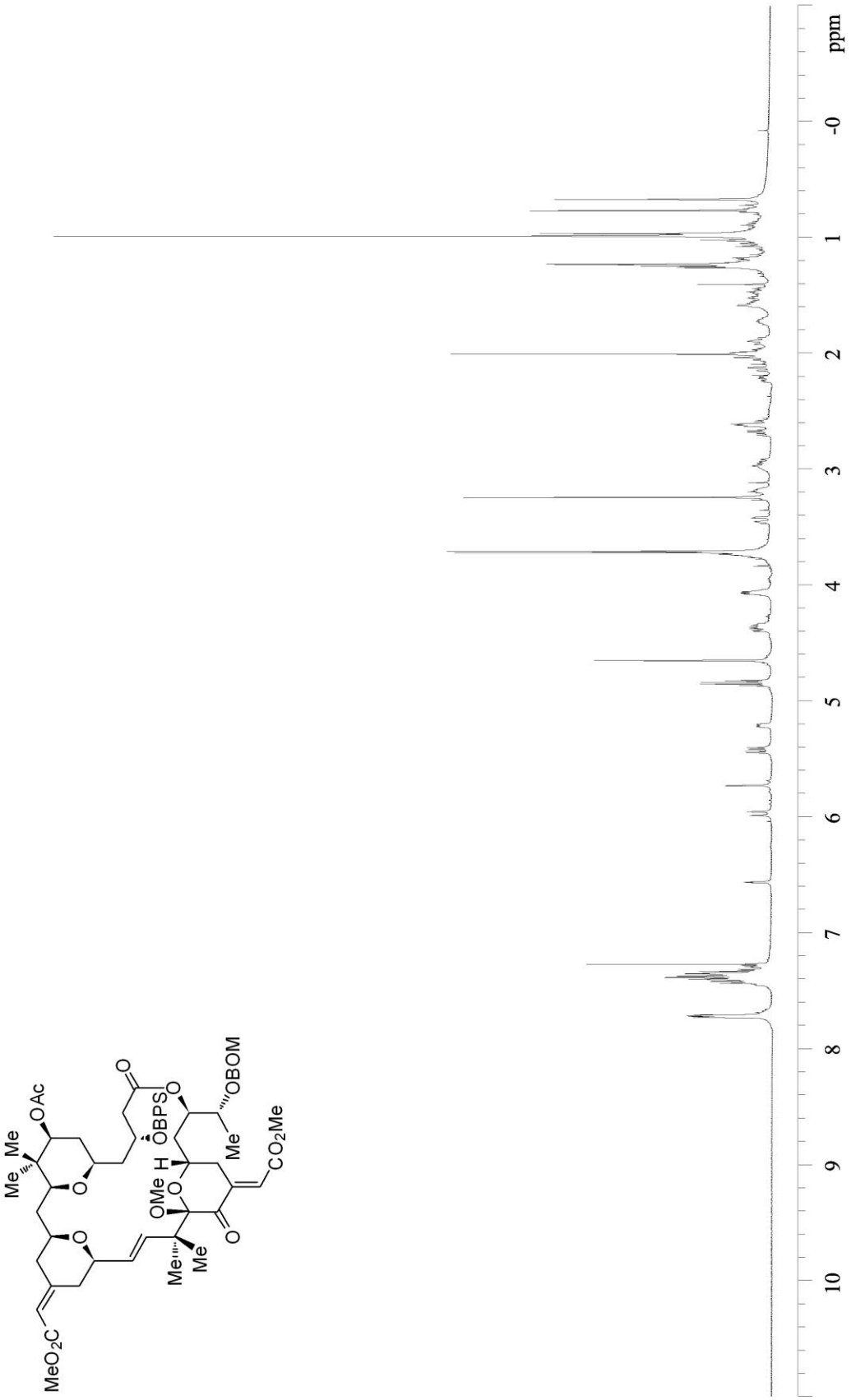

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
MeO2C
OAc
Me
Me
OMe
H
OBPS
OBOM
Me
Me
Me
200
180
160
140
120
100
80
60
40
20
0
ppm

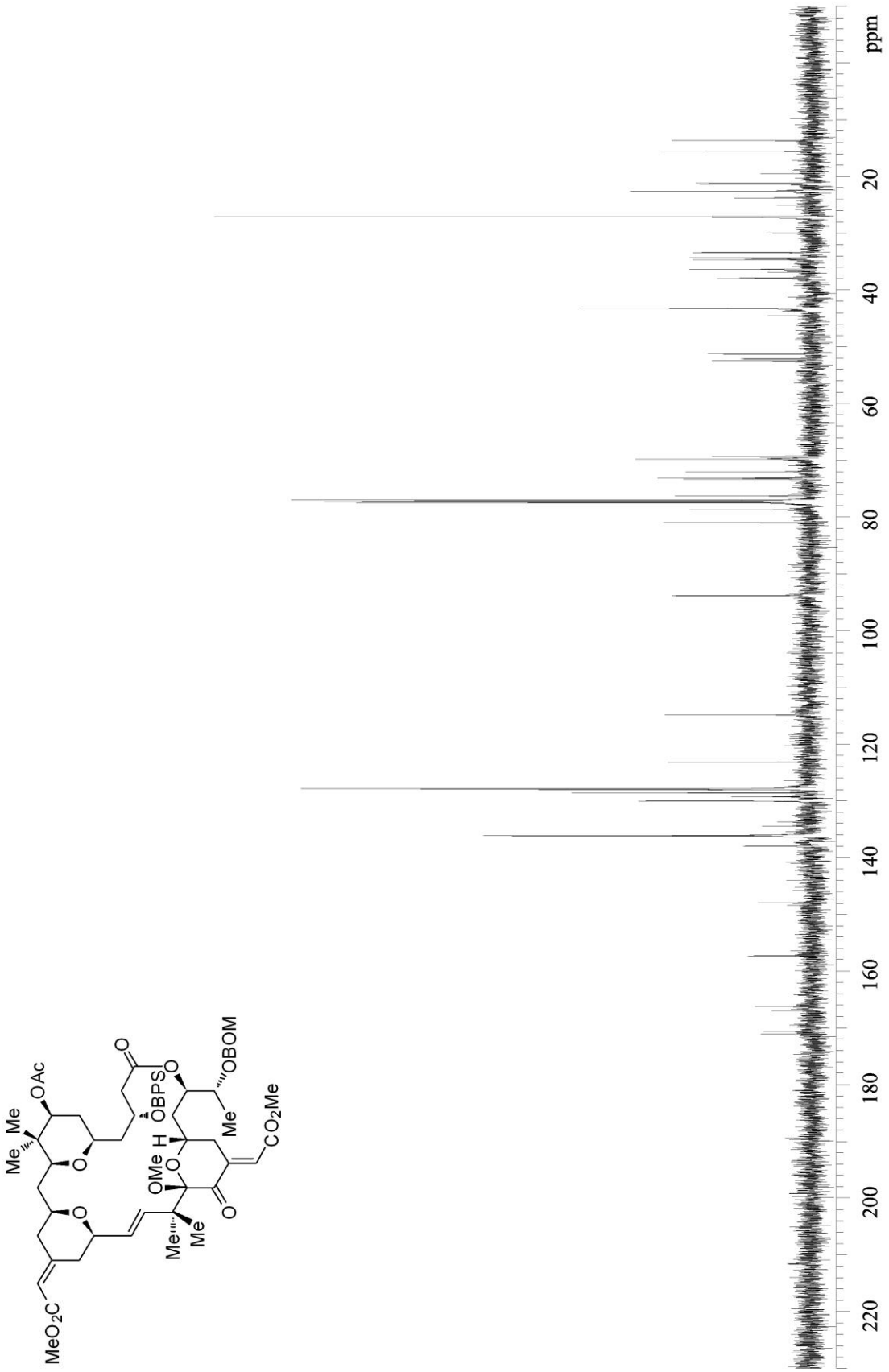

CO2Me
Me
Me
OAc
O
O
O
OMe
H
OBPS
O
Me
Me
O
O
Me
OBOM

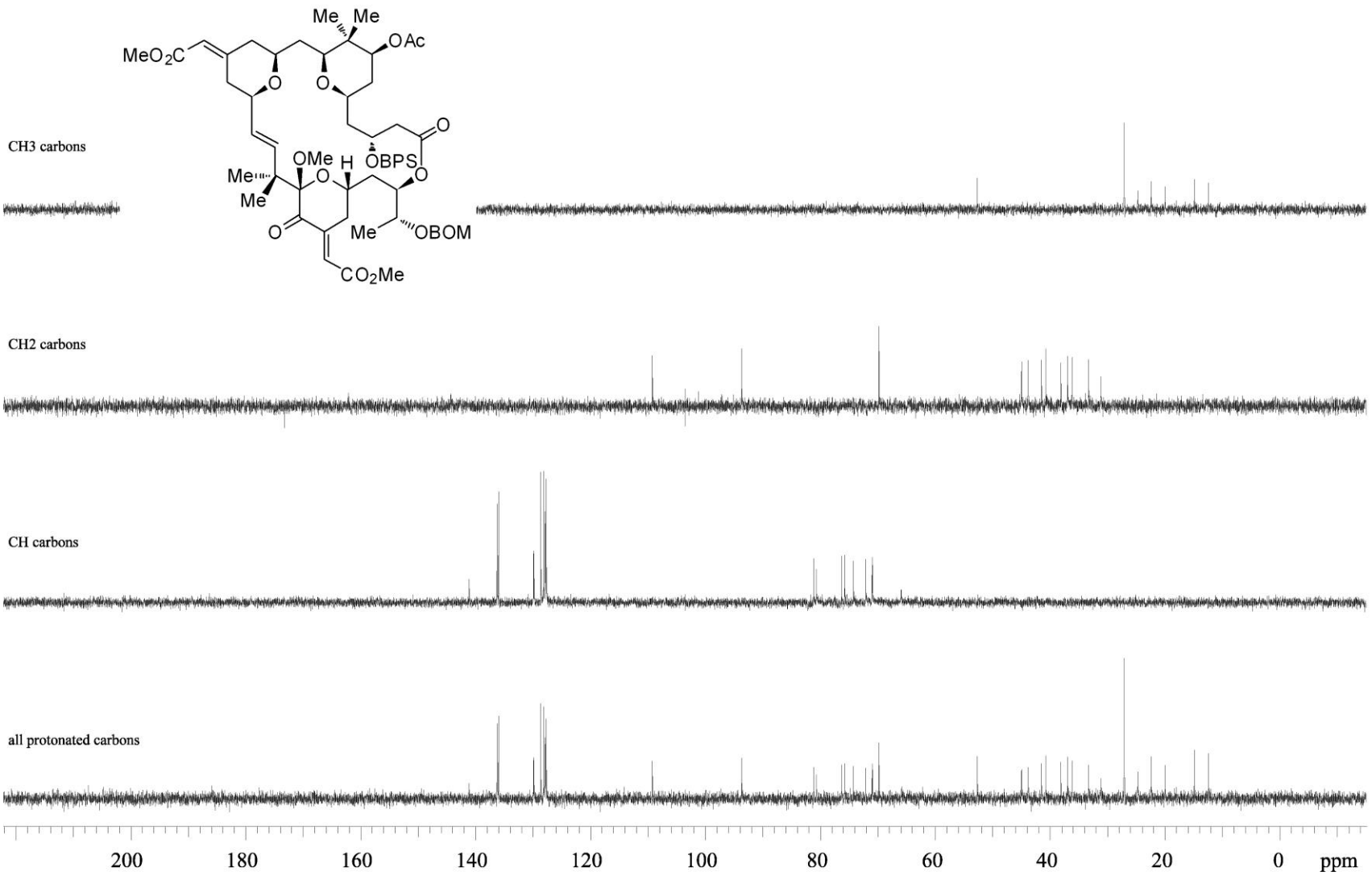
10
9
8
7
6
5
4
3
2
1
-0
ppm

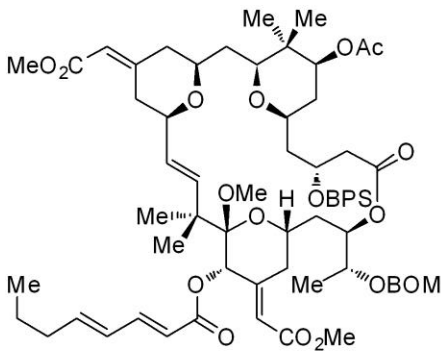

CO2Me
Me
Me
OAc
OMe
H
OBPS
Me
Me
Me
OBOM
O
O
O
O
O
O
O
O
ppm
20
40
60
80
100
120
140
160
180
200


CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
CO2Me
OAc
Me
Me
OMe
H
OBPS
Me
Me
OBOM
200
180
160
140
120
100
80
60
40
20
0
ppm

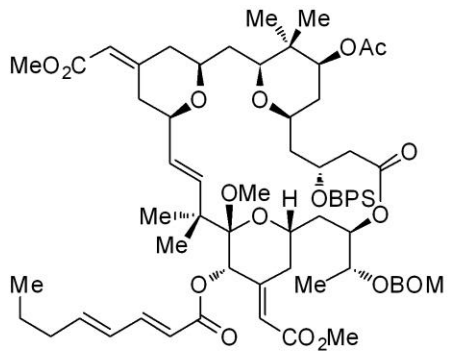

MeO2C
Me
Me
OAc
O
O
O
OMe
H
OBPS
O
Me
Me
O
O
Me
OBOM
CO2Me
ppm
10
9
8
7
6
5
4
3
2
1
-0


Me
Me
OAc
O
O
OBPS
O
H
OMe
O
O
Me
Me
Me
O
OBOM
CO2Me
MeO2C
ppm
20
40
60
80
100
120
140
160
180
200
220

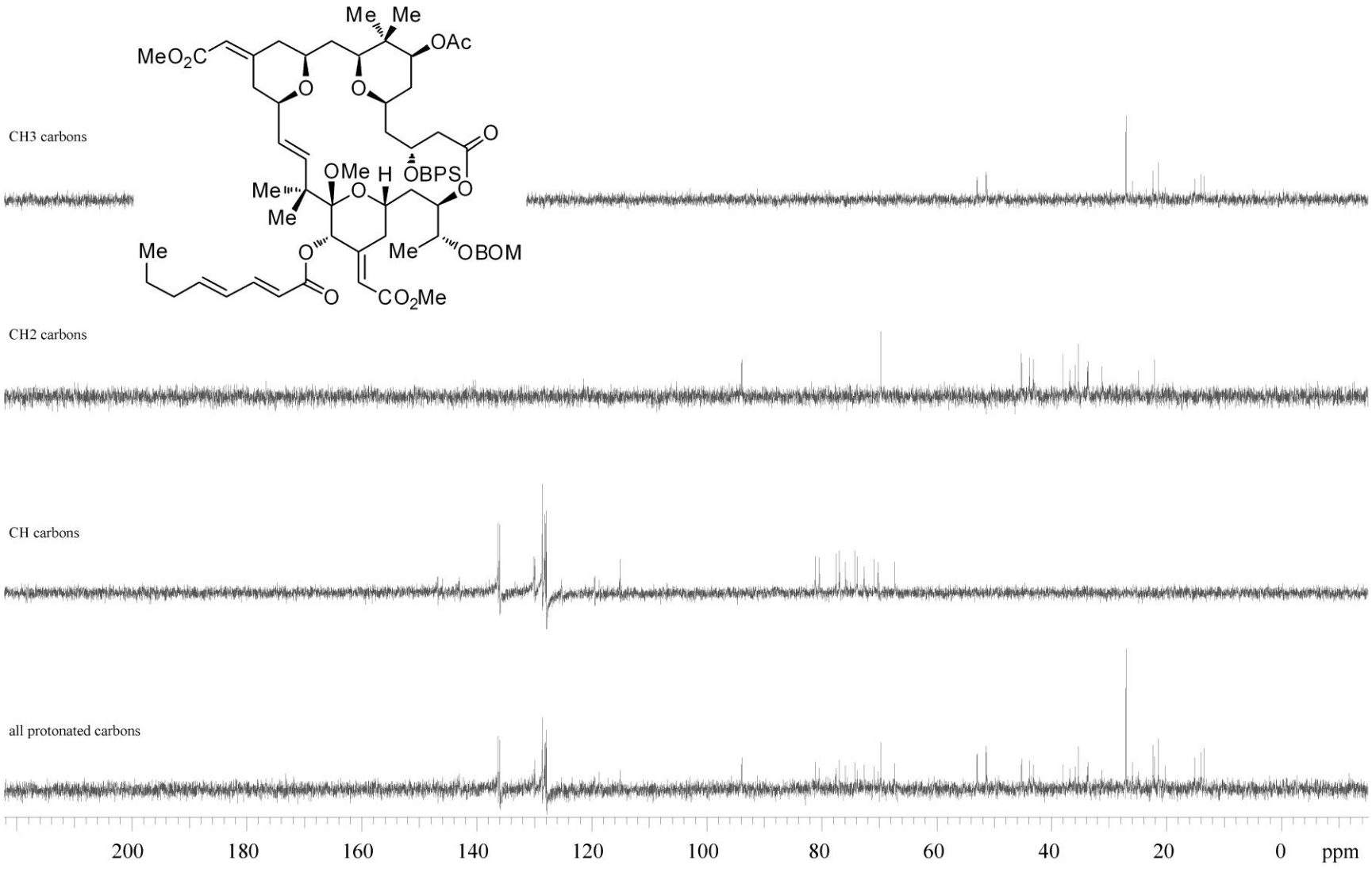

Me Me
OAc
MeO2C
O
O
O
OMe H OBPS
O
Me
Me
O
Me
OBOM
CO2Me
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

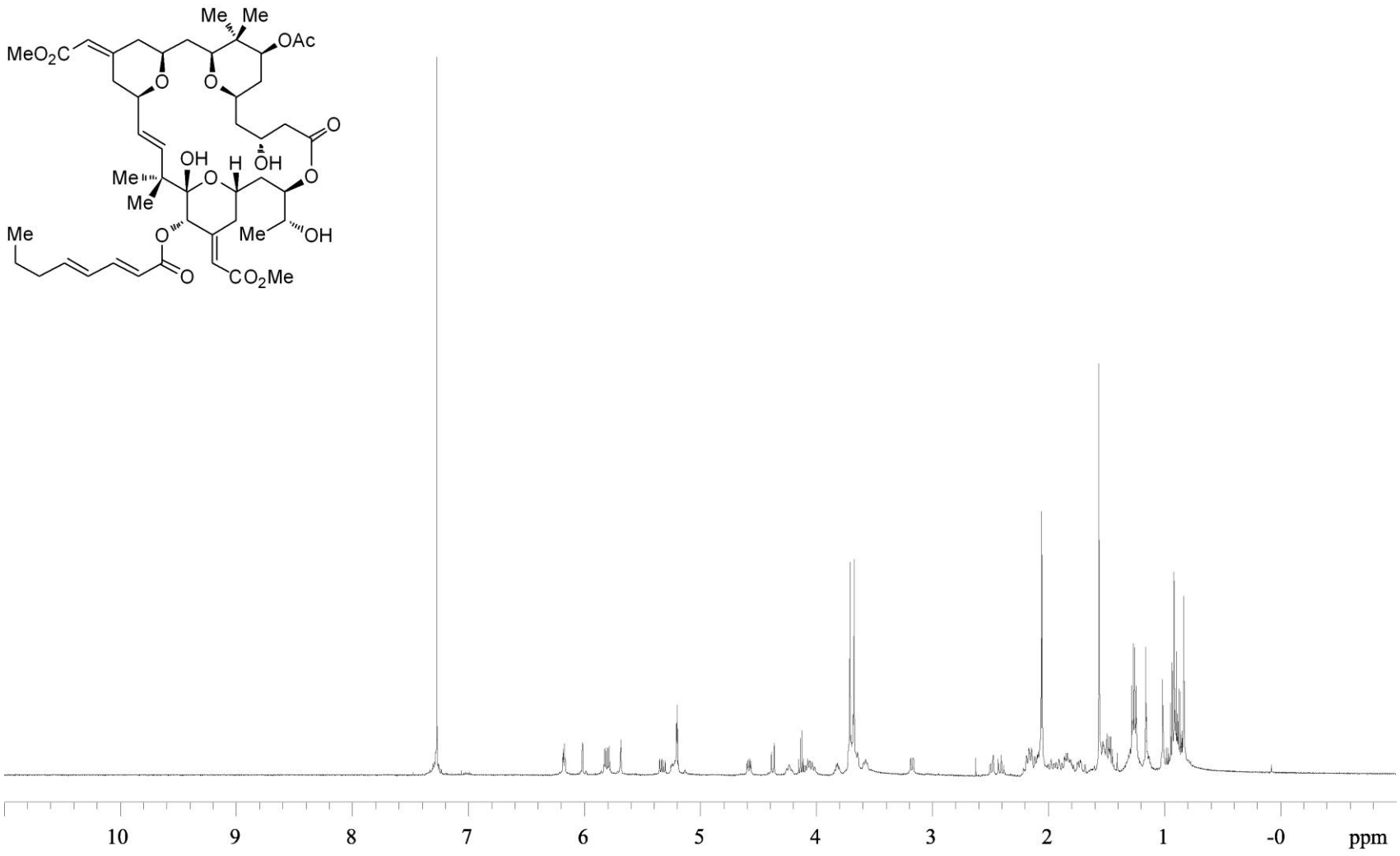

Me
Me
MeO2C
OAc
O
O
O
OMe
H
OBPS
O
Me
Me
O
Me
OBOM
Me
O
O
CO2Me

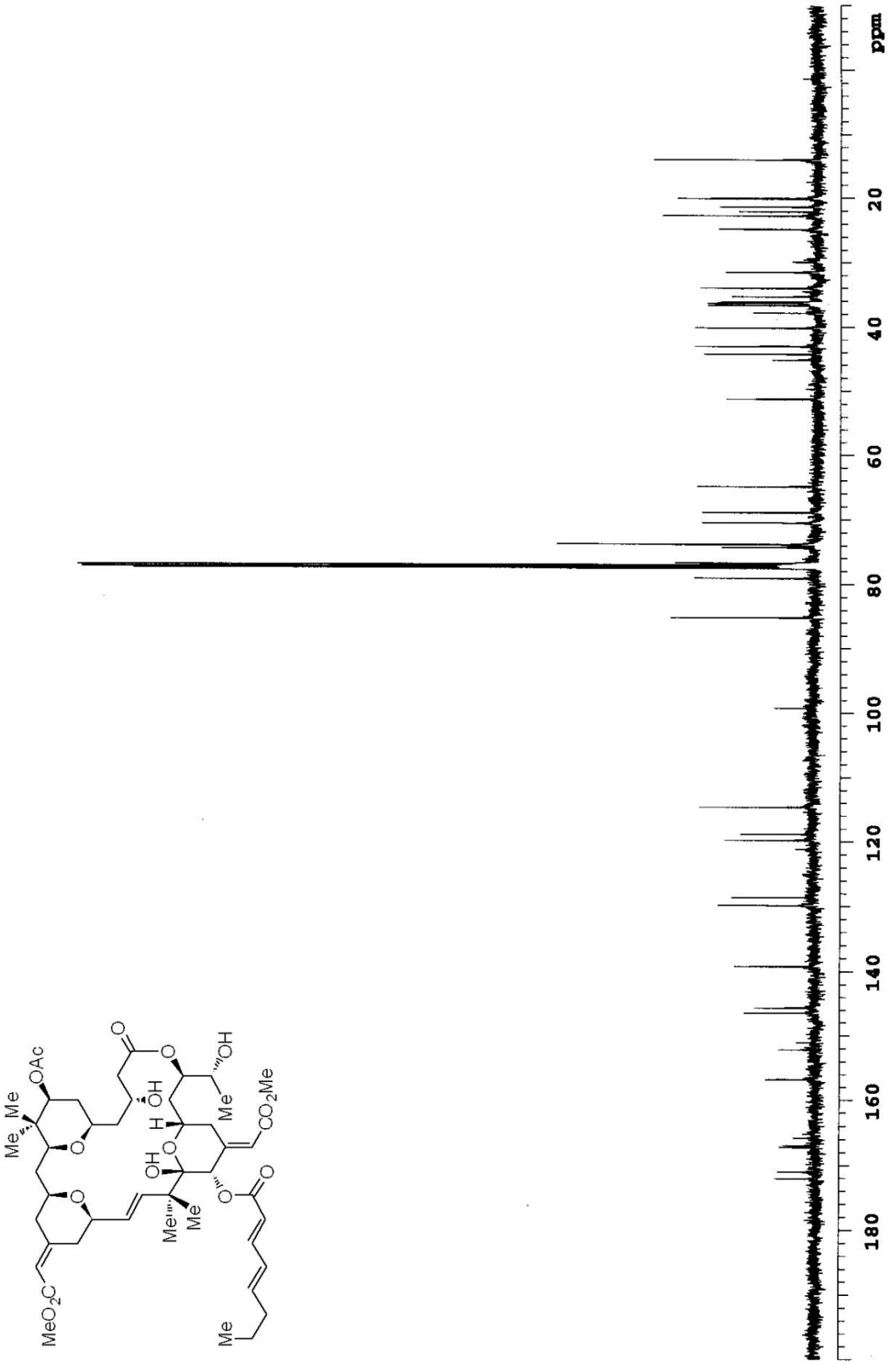
10
9
8
7
6
5
4
3
2
1
-0
ppm

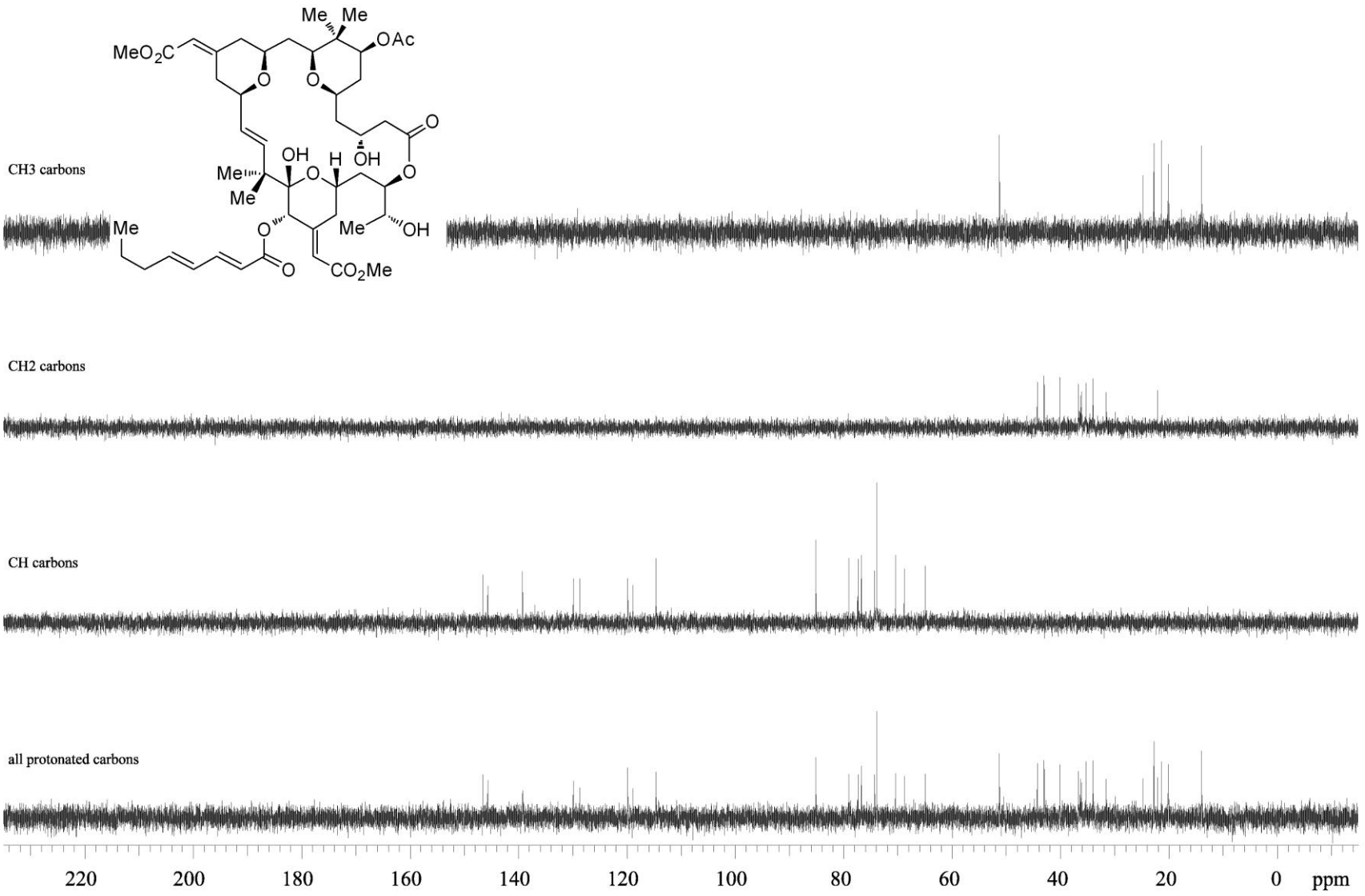

Me
Me
OAc
MeO2C
O
O
O
OMe
H
OBPS
O
Me
Me
O
Me
OBOM
Me
O
O
CO2Me

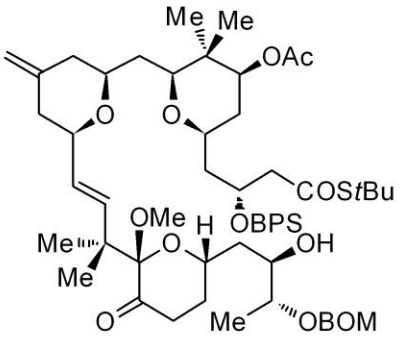
200
180
160
140
120
100
80
60
40
20
ppm


CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
Me
Me
OAc
MeO2C
O
O
O
OMe
H
OBPS
O
Me
Me
Me
O
Me
OBOM
O
CO2Me
200
180
160
140
120
100
80
60
40
20
0
ppm

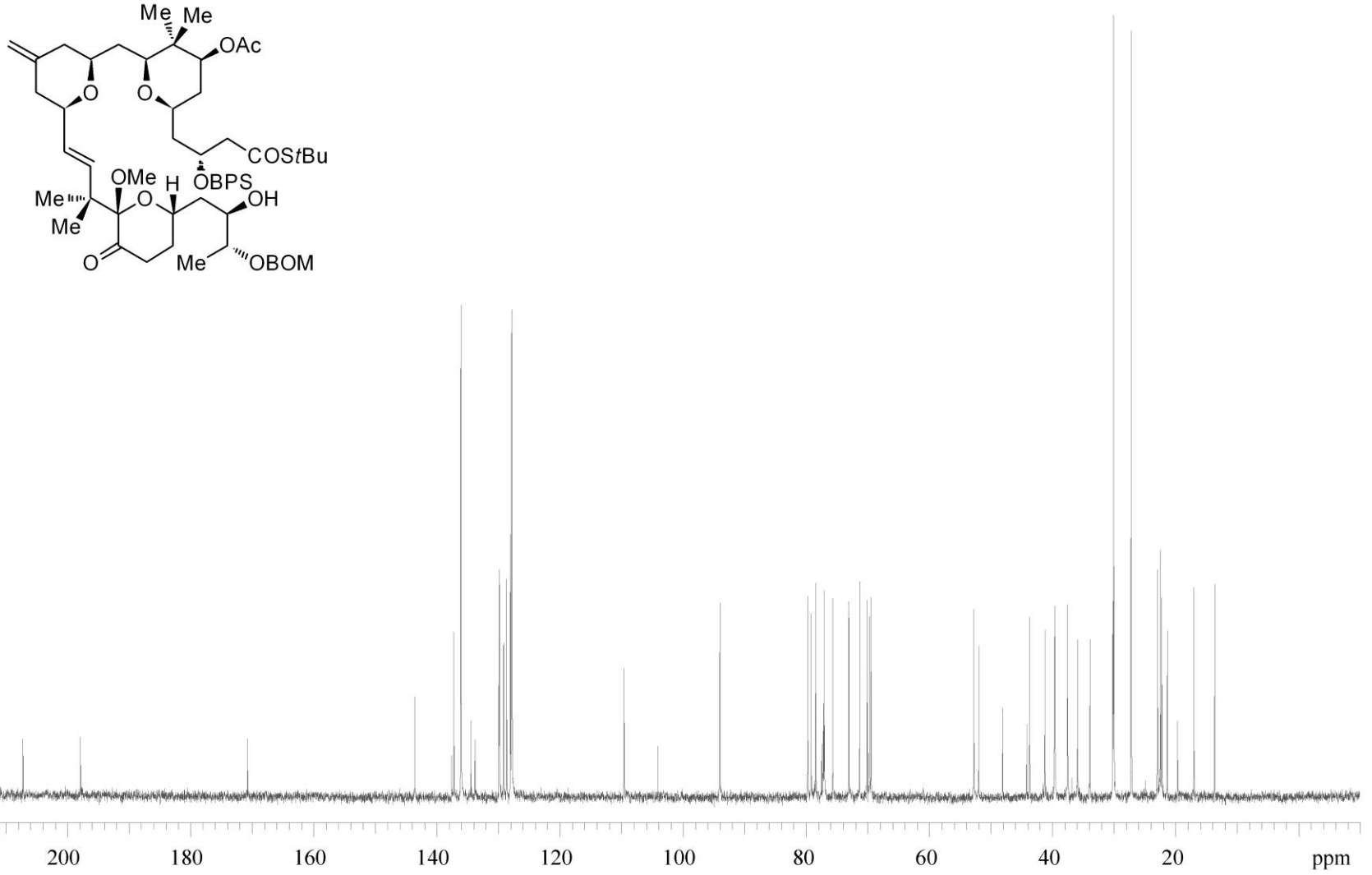

Me
Me
MeO2C
OAc
O
O
O
OH
H
OH
O
O
Me
Me
Me
O
Me
OH
O
CO2Me
10
9
8
7
6
5
4
3
2
1
-0
ppm

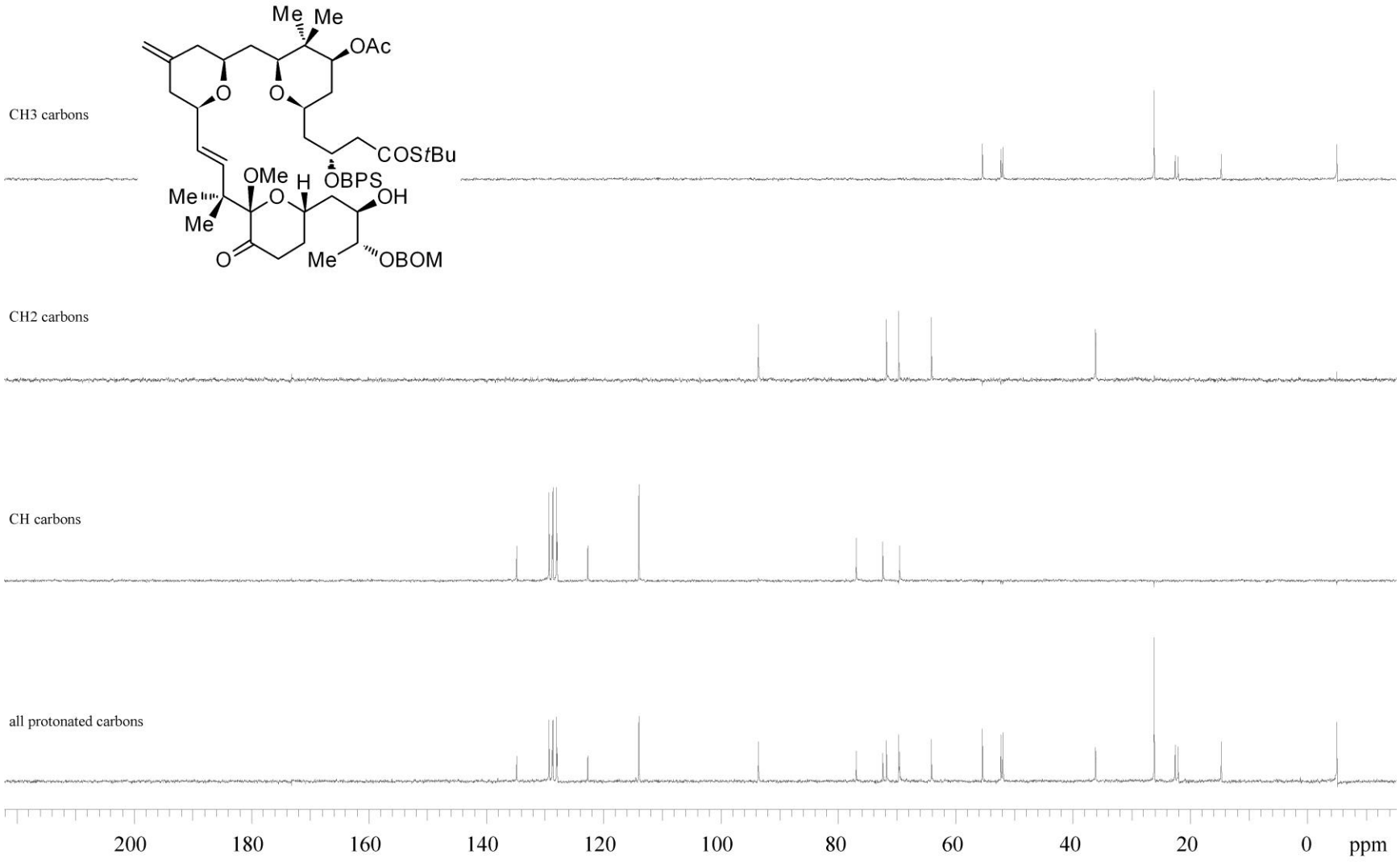

Me
Me
OAc
O
O
O
H
OH
OH
O
Me
Me
Me
OH
CO2Me
O
O
MeO2C
Me
ppm
20
40
60
80
100
120
140
160
180

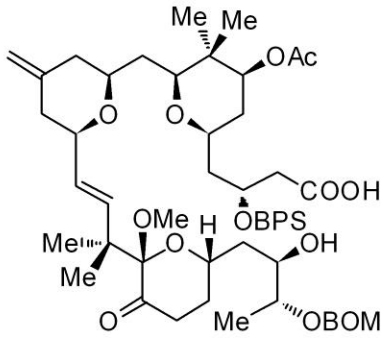

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
220
200
180
160
140
120
100
80
60
40
20
0
ppm


Me
Me
OAc
O
O
COS*t*Bu
OBPS
OMe
H
O
OH
Me
Me
O
Me
OBOM

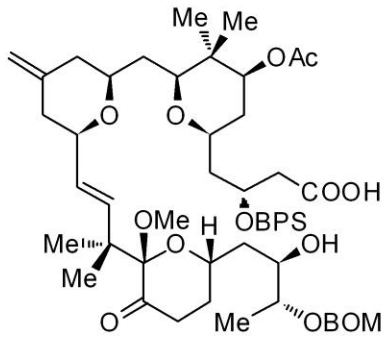
10
9
8
7
6
5
4
3
2
1
-0
ppm


Me
Me
OAc
O
O
COS*t*Bu
OMe
H
OBPS
Me
O
OH
Me
O
Me
OBOM
200
180
160
140
120
100
80
60
40
20
ppm

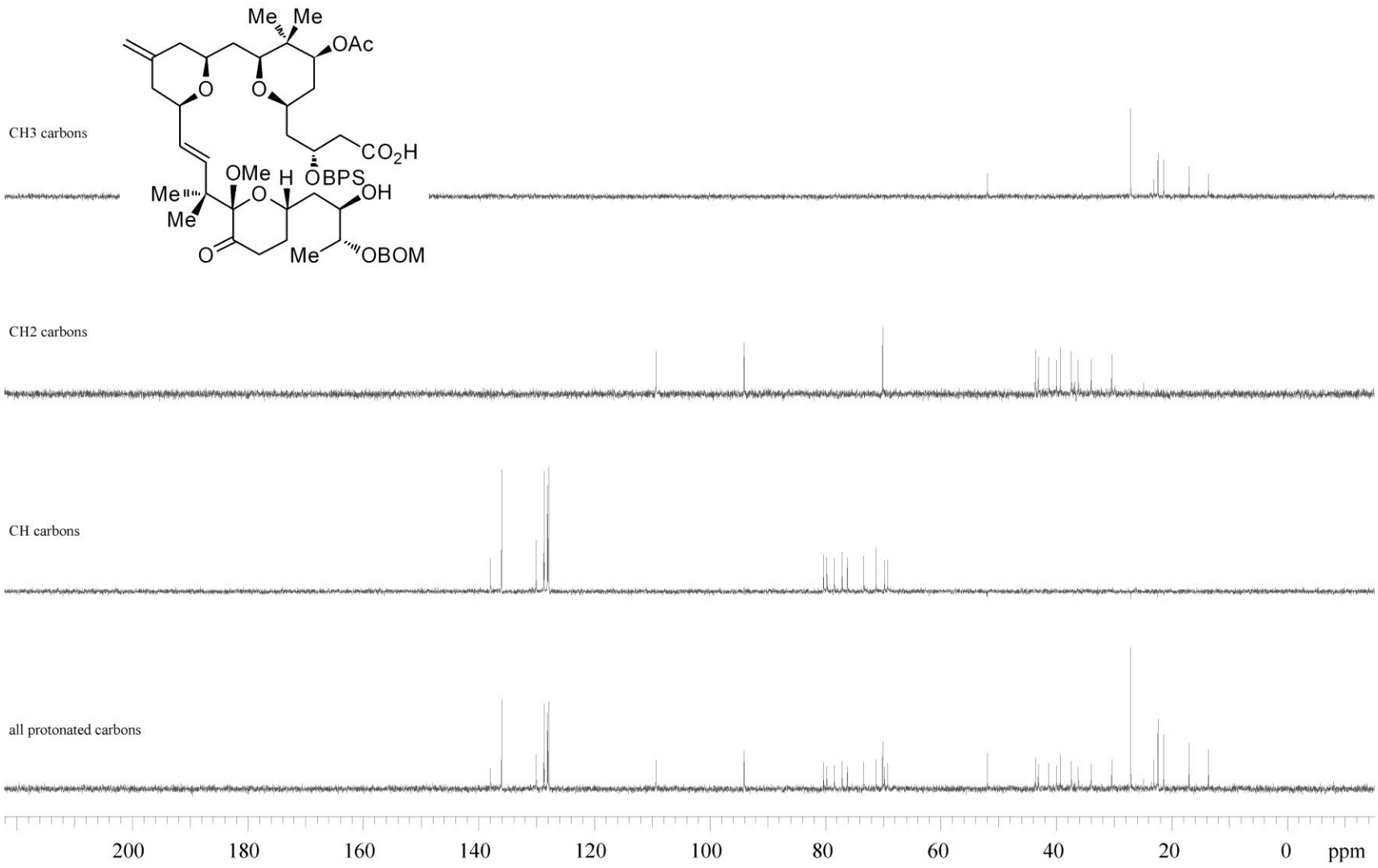

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
Me
Me
OAc
O
O
COS*t*Bu
OMe
H
OBPS
Me
Me
O
OH
O
Me
OBOM
200
180
160
140
120
100
80
60
40
20
0
ppm

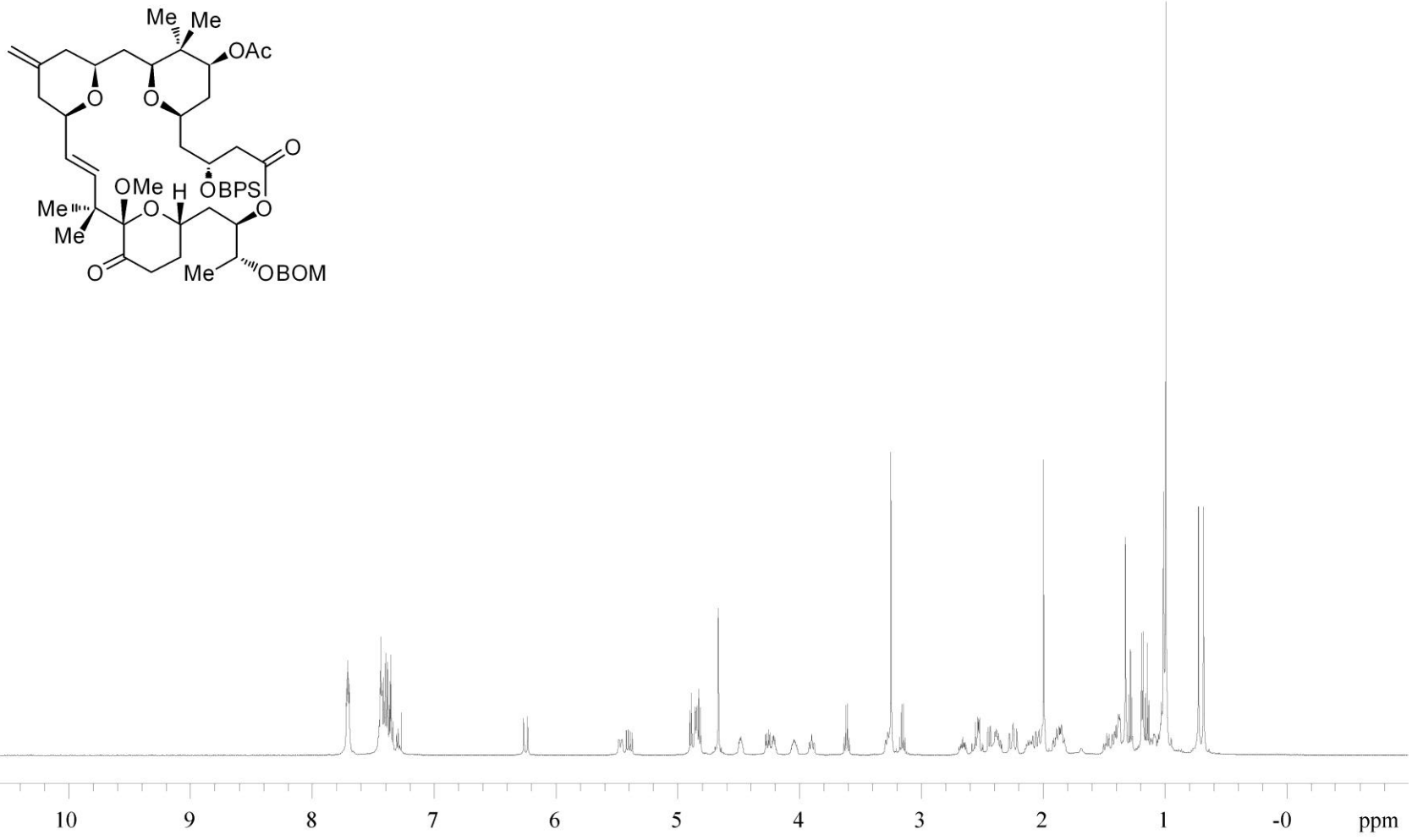

Me
Me
OAc
O
O
COOH
OMe
H
OBPS
Me
O
OH
Me
O
Me
OBOM

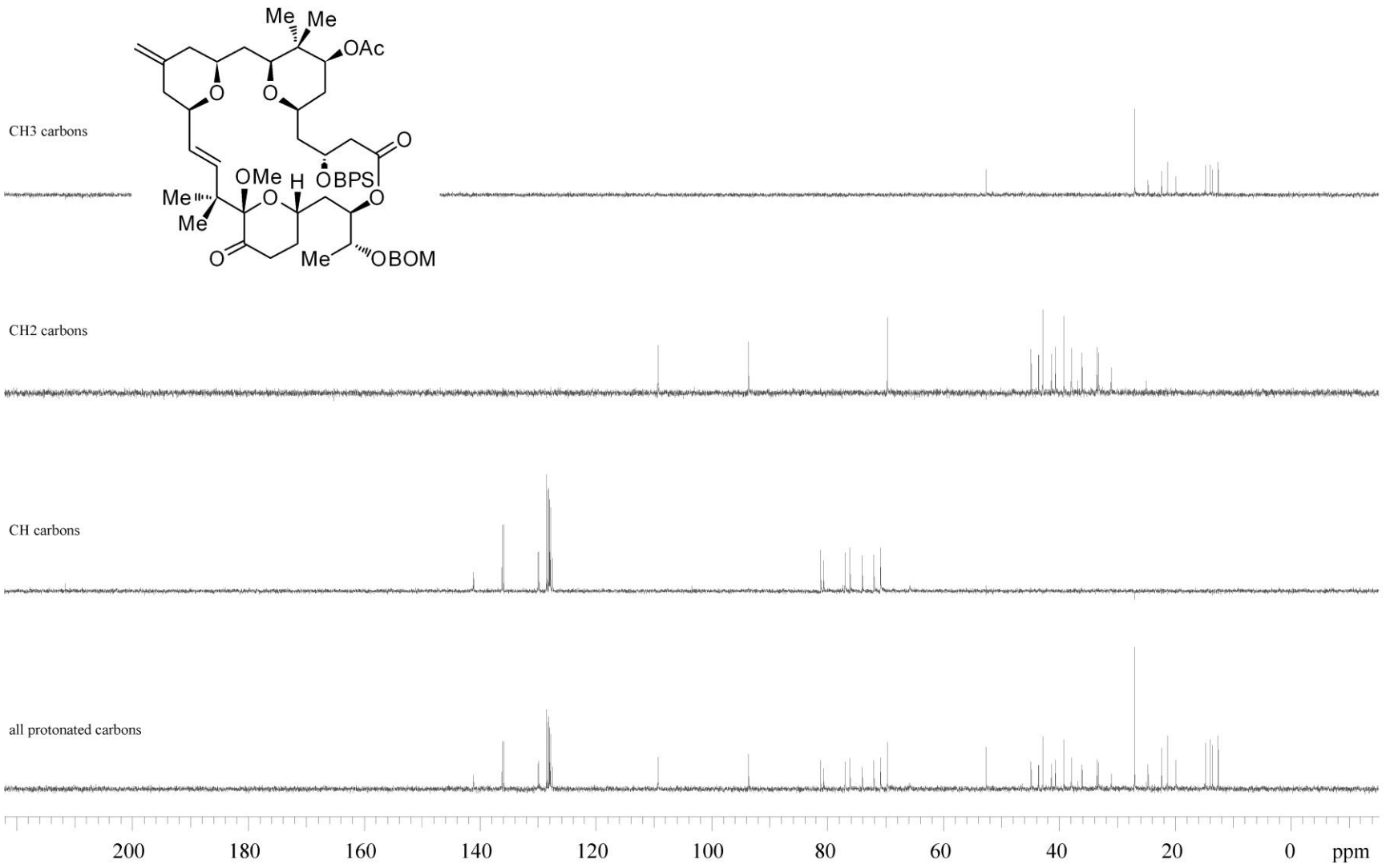
10
9
8
7
6
5
4
3
2
1
-0
ppm

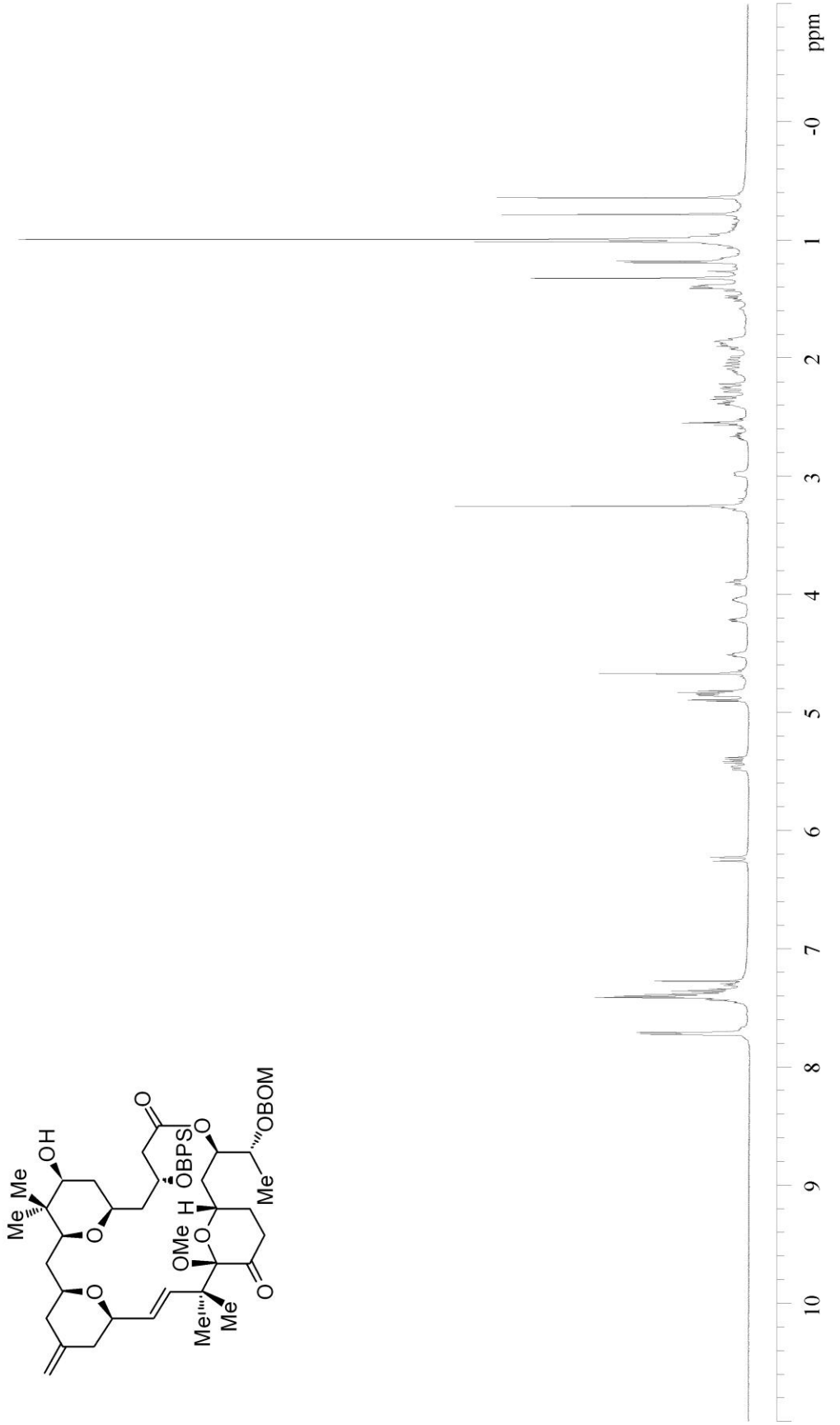

Me
Me
OAc
O
O
COOH
OMe
H
OBPS
Me
O
OH
Me
O
Me
OBOM

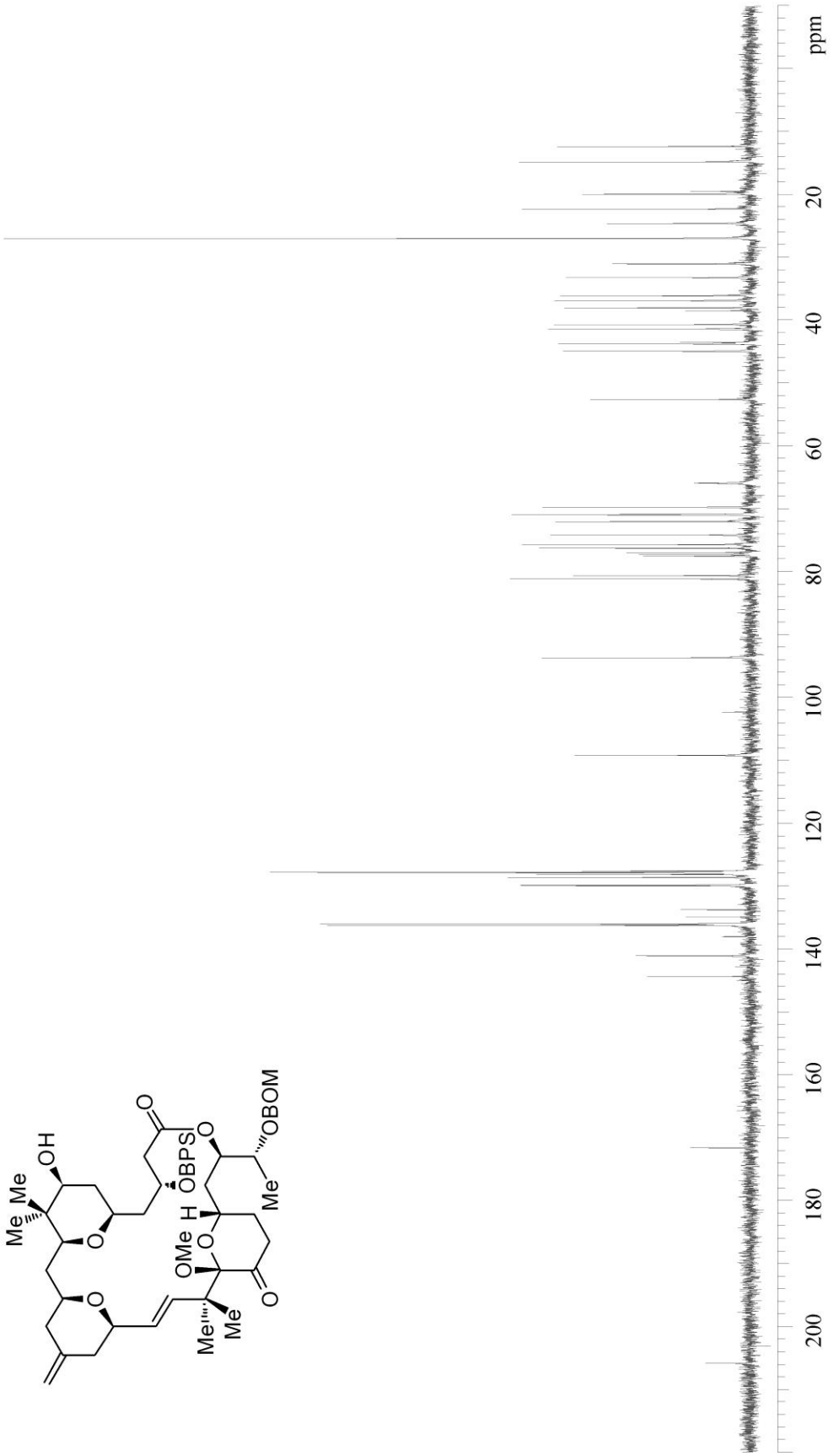
200
180
160
140
120
100
80
60
40
20
ppm

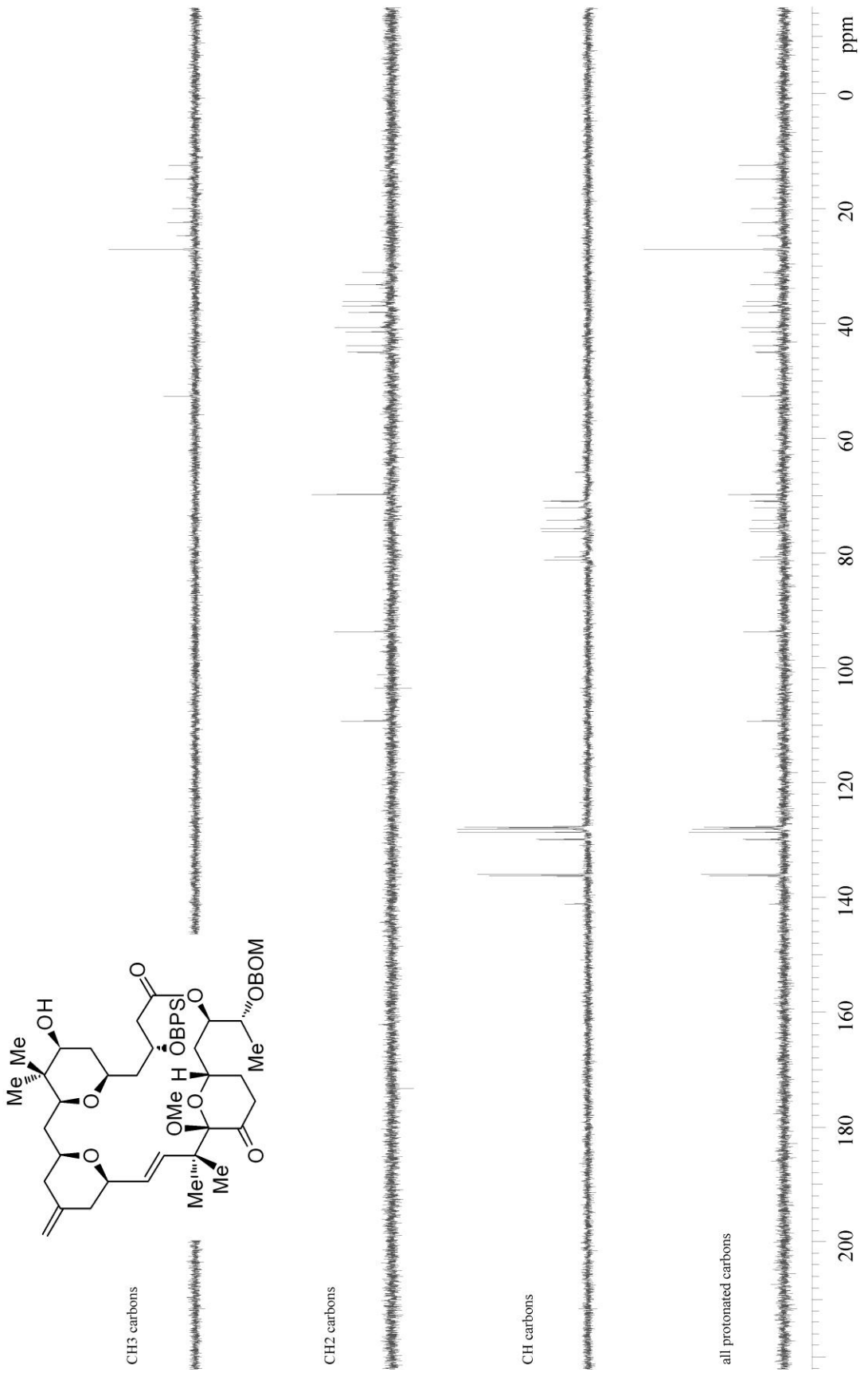

Me Me
OAc
O
O
CO2H
OMe H OBPS
Me
Me
OH
O
O
Me
OBOM
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

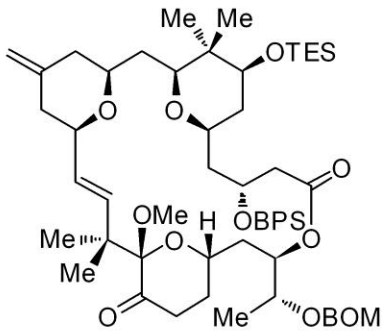

Me
Me
OAc
O
O
O
OMe
H
OBPS
Me
Me
O
O
O
Me
OBOM
10
9
8
7
6
5
4
3
2
1
-0
ppm


Me Me
OAc
O
O
O
OMe
H
OBPS
O
Me
Me
O
O
Me
OBOM
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

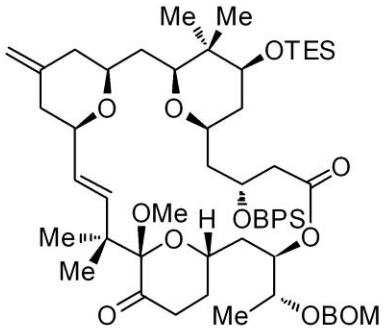

Me
Me
OH
O
O
OMe
H
OBPSI
O
O
Me
Me
O
O
Me
OBOM
ppm
10
9
8
7
6
5
4
3
2
1
-0


Me
Me
OH
O
O
O
OBPS
H
OMe
O
O
Me'''
Me
Me
OBOM
ppm
200
180
160
140
120
100
80
60
40
20

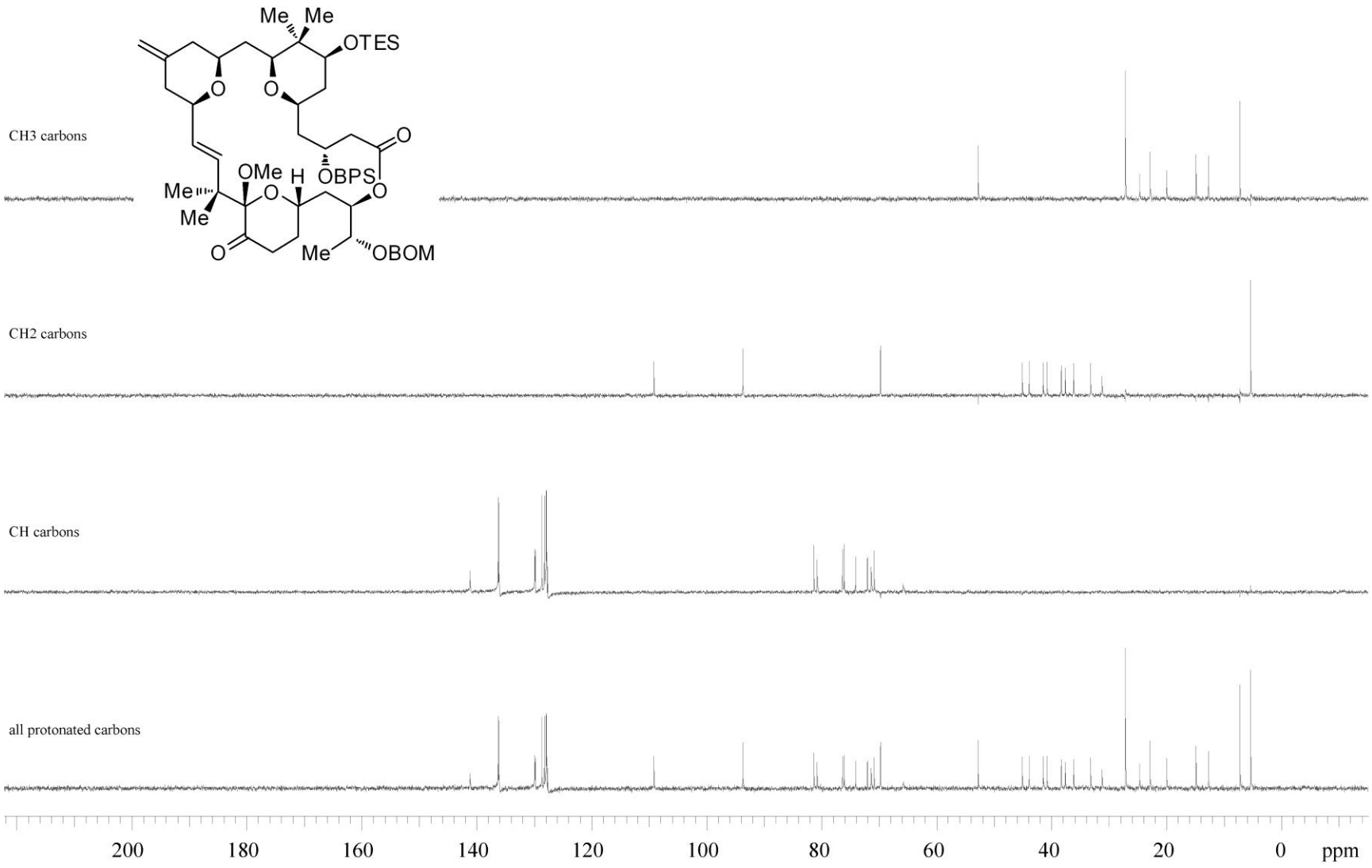

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
Me
Me
OH
OMe
H
OBPS
Me
OBOM
Me
Me
O
200
180
160
140
120
100
80
60
40
20
0
ppm

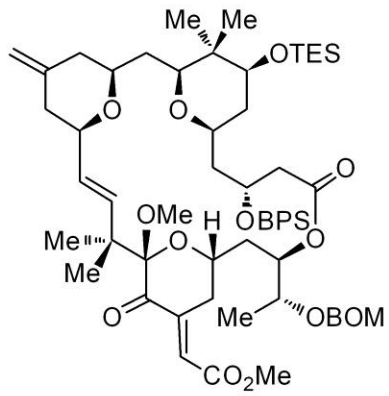

Me
Me
OTES
O
O
O
OMe
H
OBPS
O
Me
Me
O
Me
OBOM

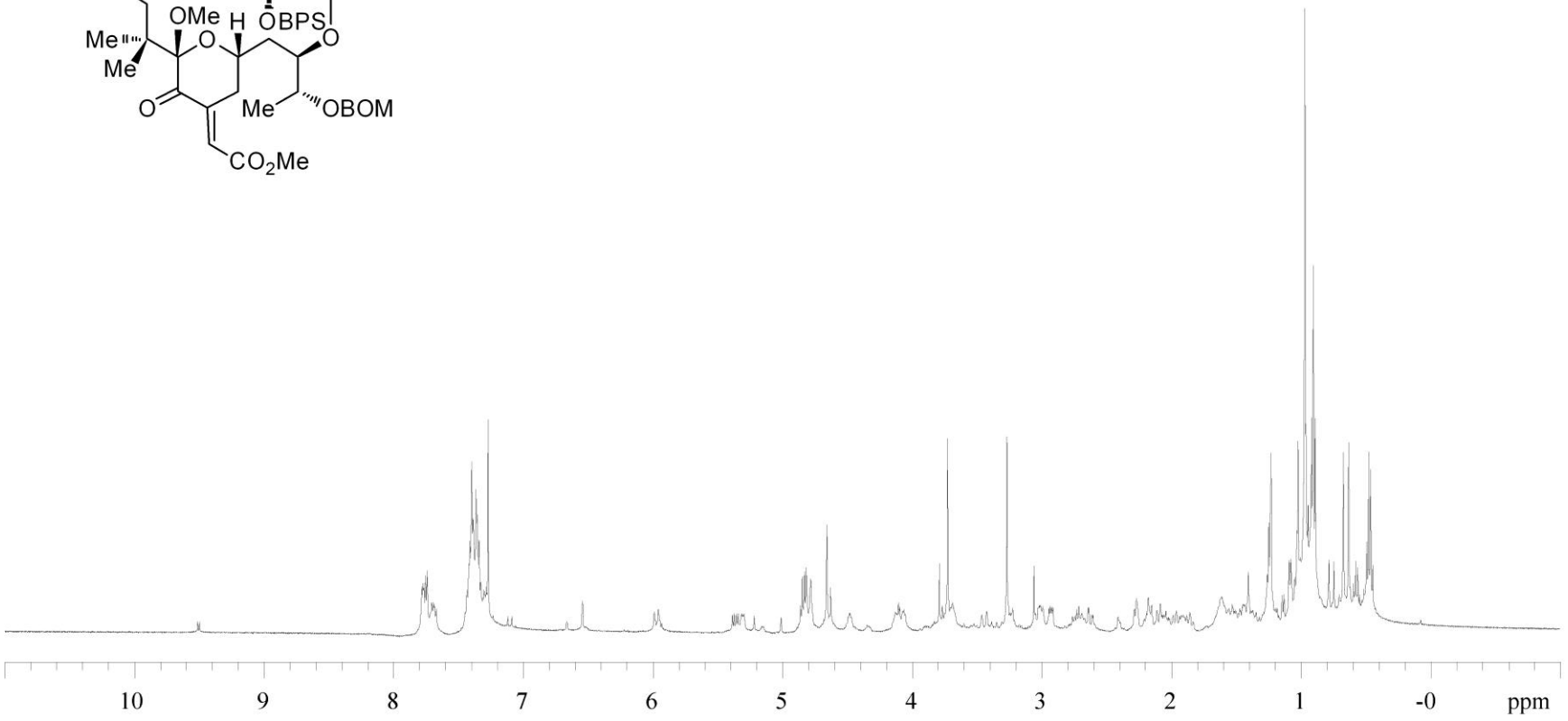
10
9
8
7
6
5
4
3
2
1
-0
ppm

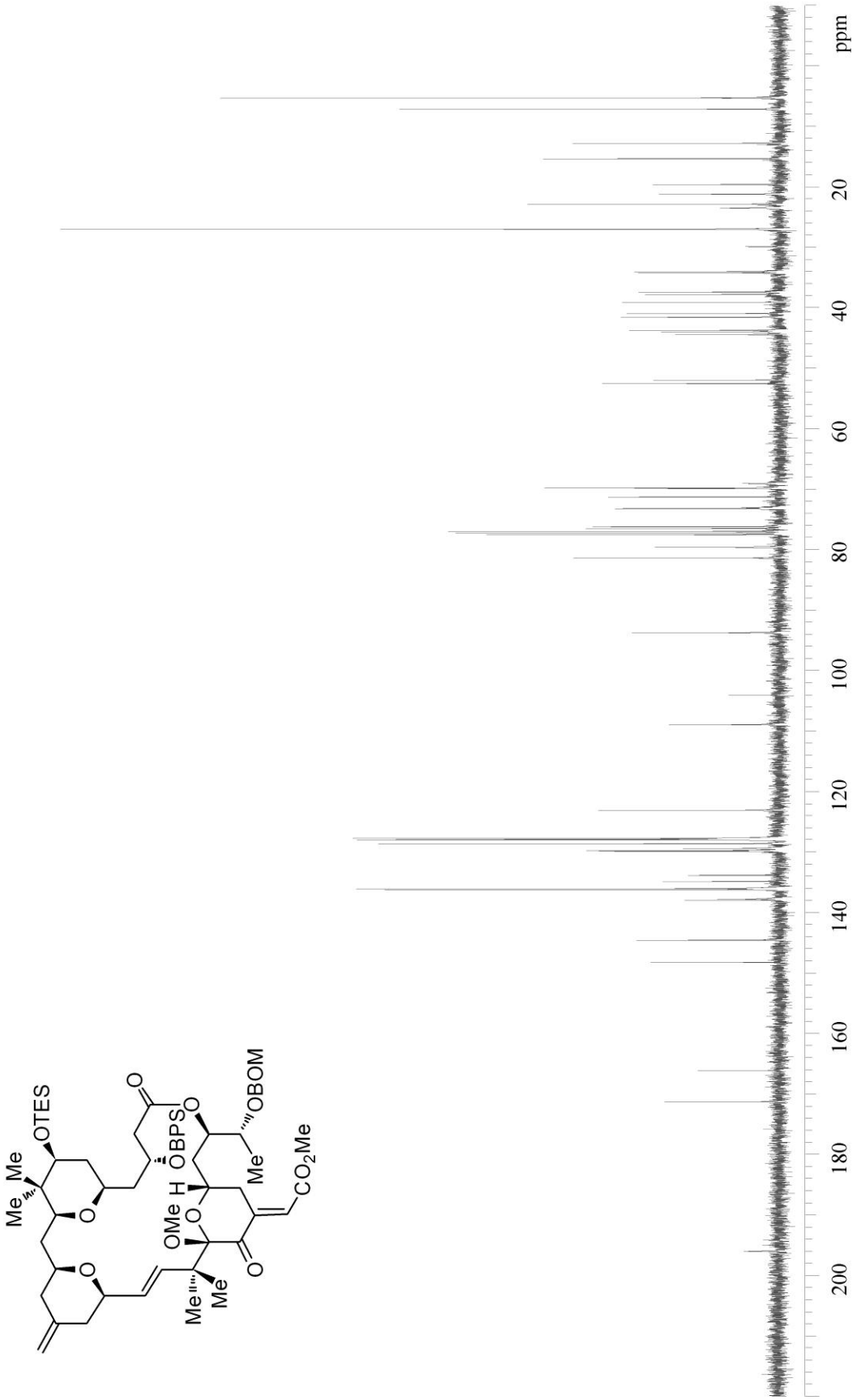

Me
Me
OTES
O
O
O
OMe
H
OBPS
O
Me
Me
O
Me
OBOM

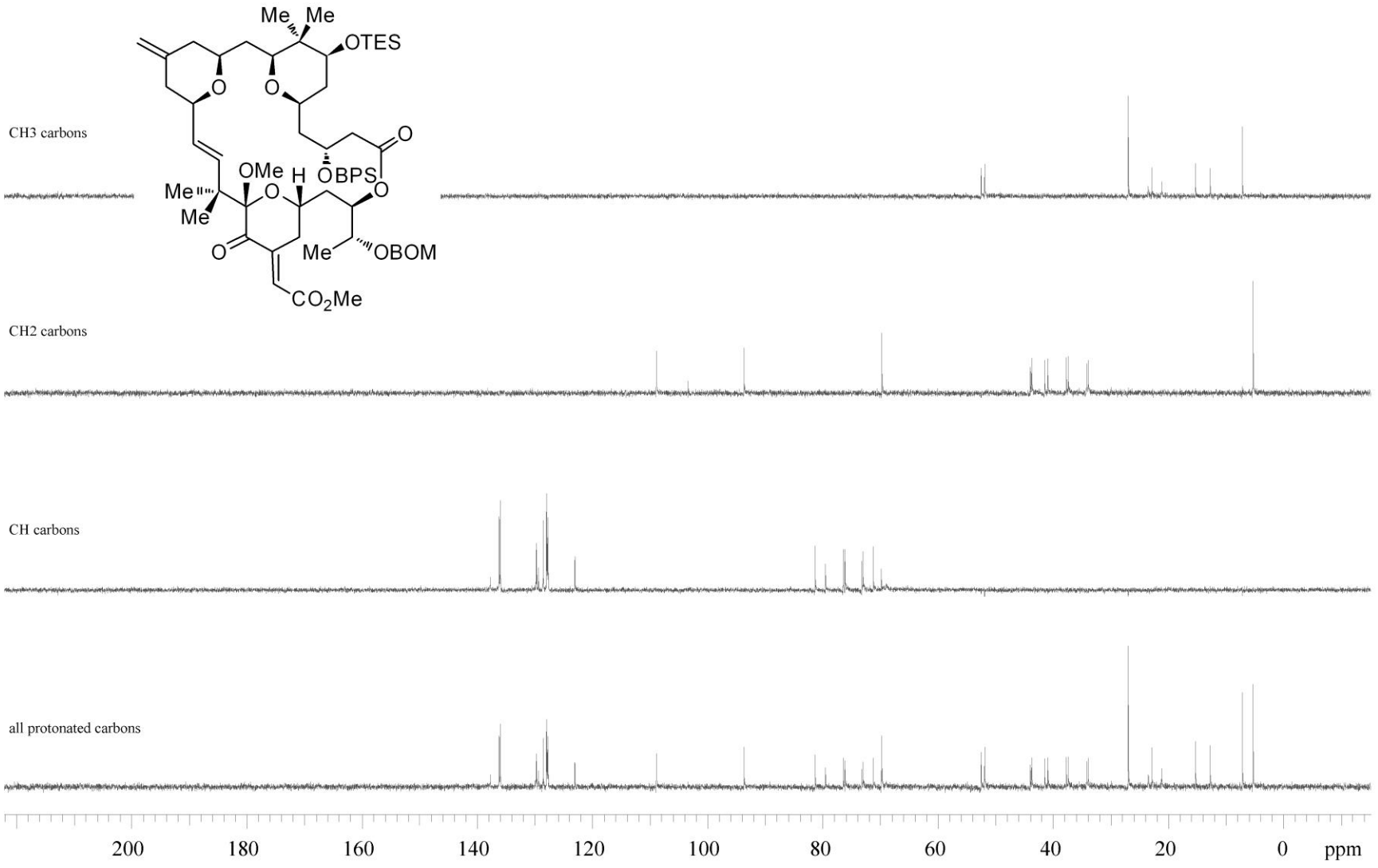
200
180
160
140
120
100
80
60
40
20
ppm

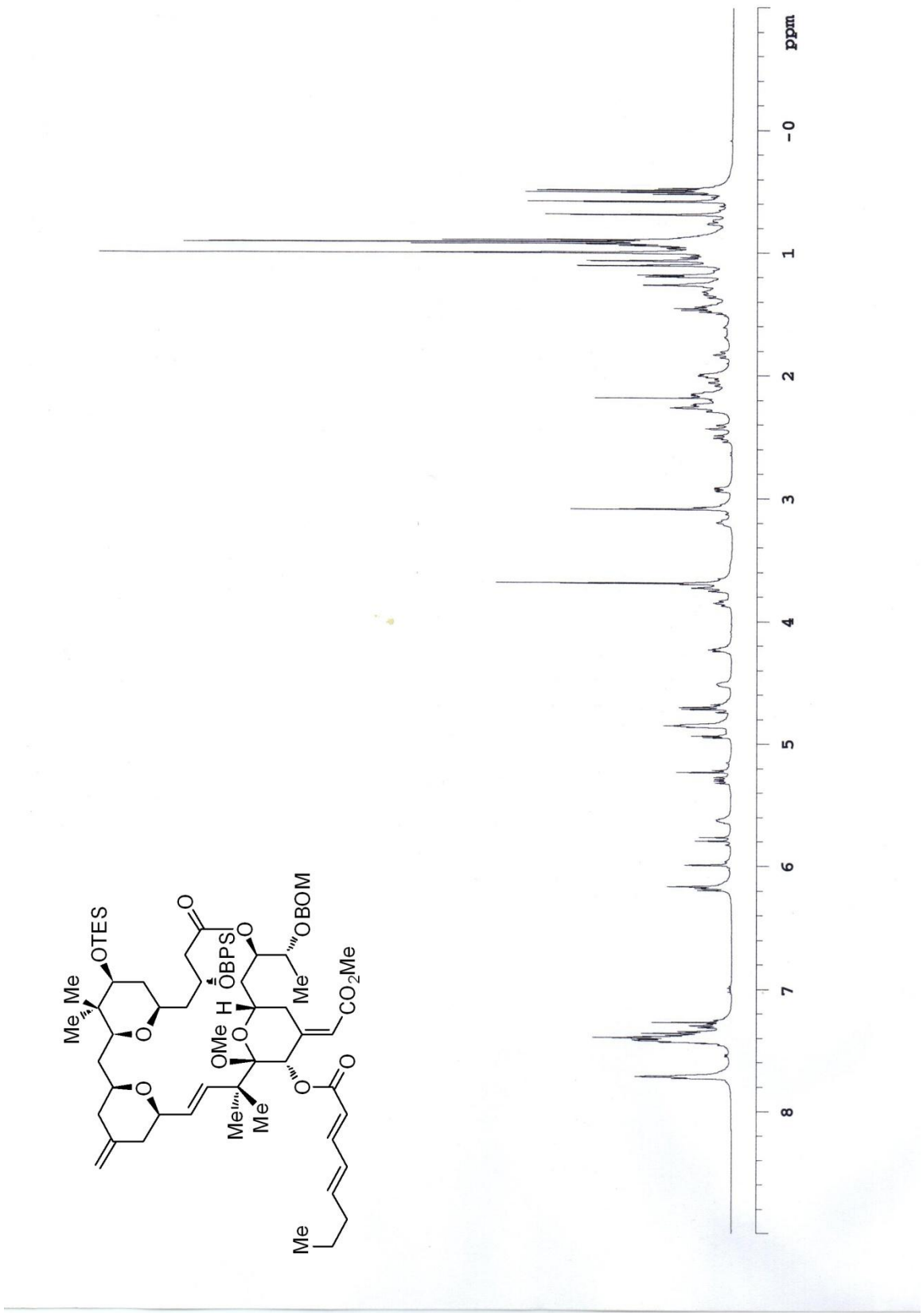

Me Me
OTES
O
O
O
OMe H OBPS
O
Me
Me
O
Me
OBOM
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

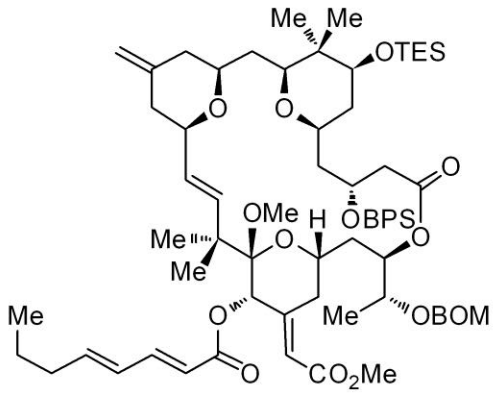

Me
Me
OTES
O
O
O
OMe
H
OBPS
O
Me
Me
O
O
Me
OBOM
CO2Me


10
9
8
7
6
5
4
3
2
1
-0
ppm

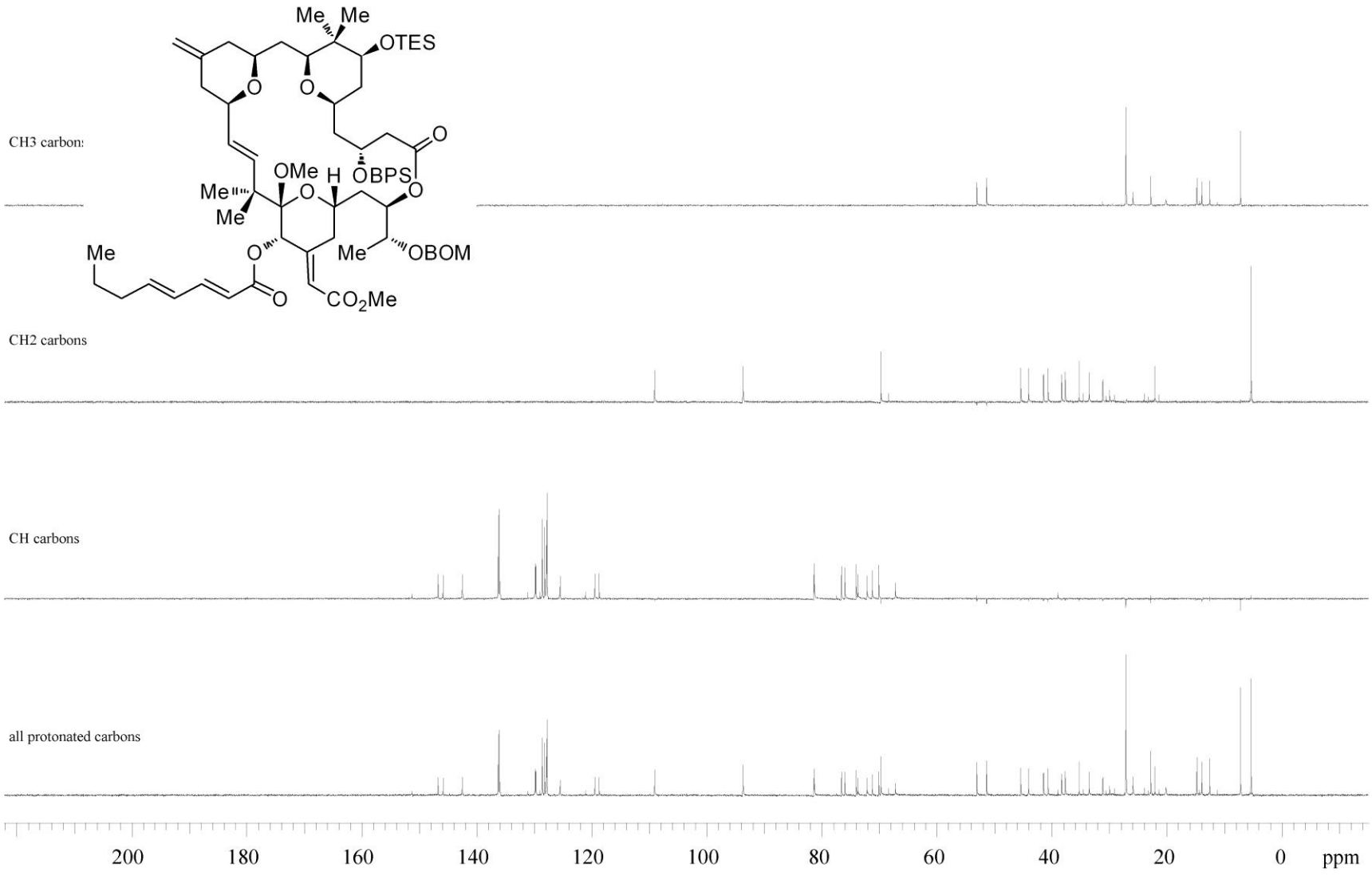

Me
Me
OTES
O
O
OMe
H
OBPS
O
O
Me
Me
O
O
Me
OBOM
CO2Me
ppm
200
180
160
140
120
100
80
60
40
20

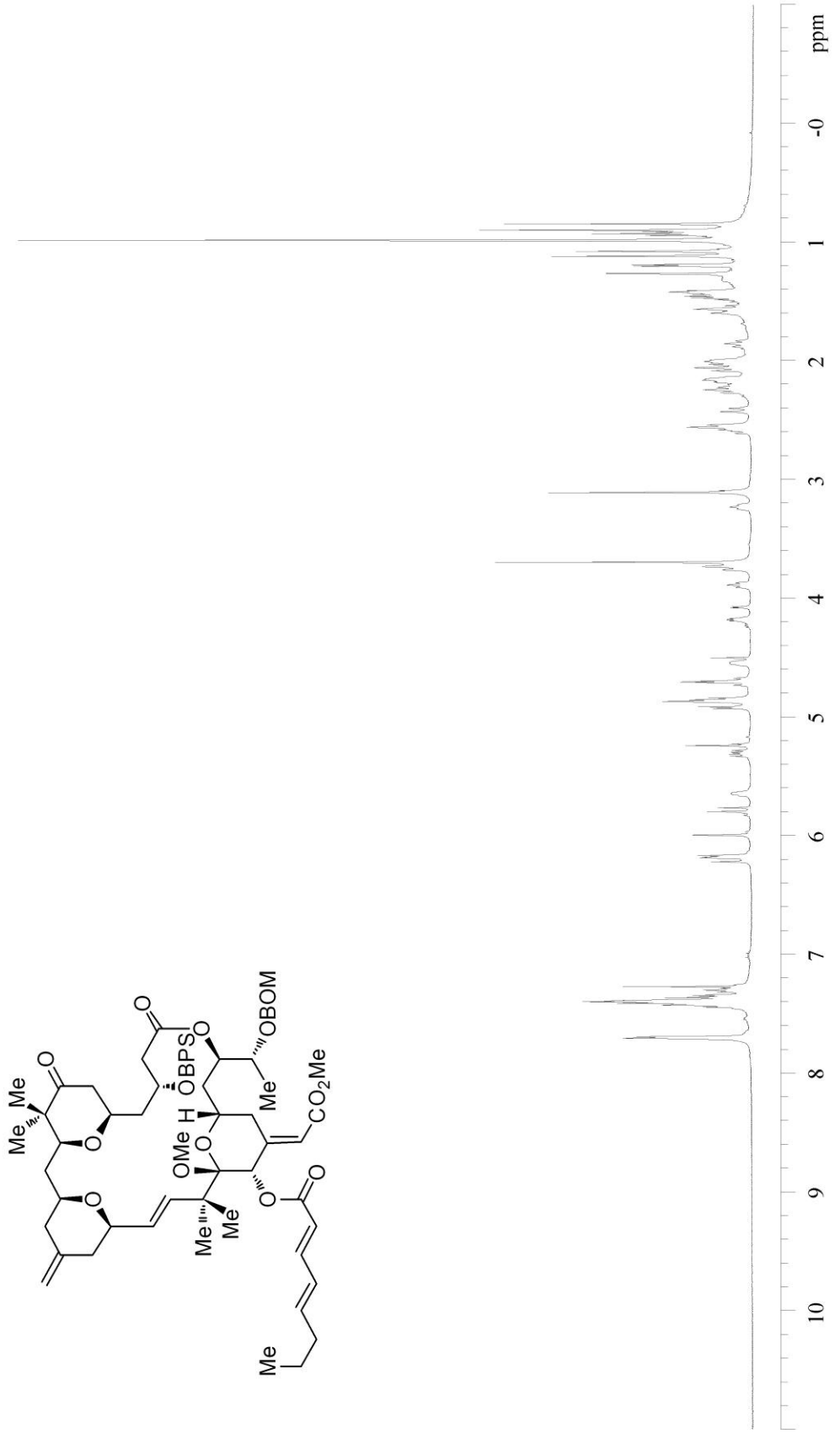

Me Me
OTES
O
O
O
OMe
H
OBPS
O
Me
Me
O
Me
OBOM
CO2Me
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

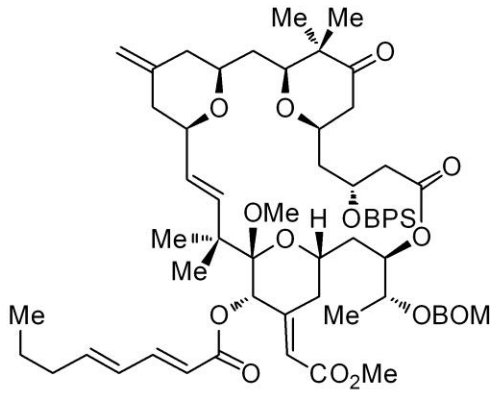

Me
Me
OTES
O
O
OMe
H
OBPS
O
O
Me
Me
Me
OBOM
CO2Me
O
O
Me
8
7
6
5
4
3
2
1
-0
ppm

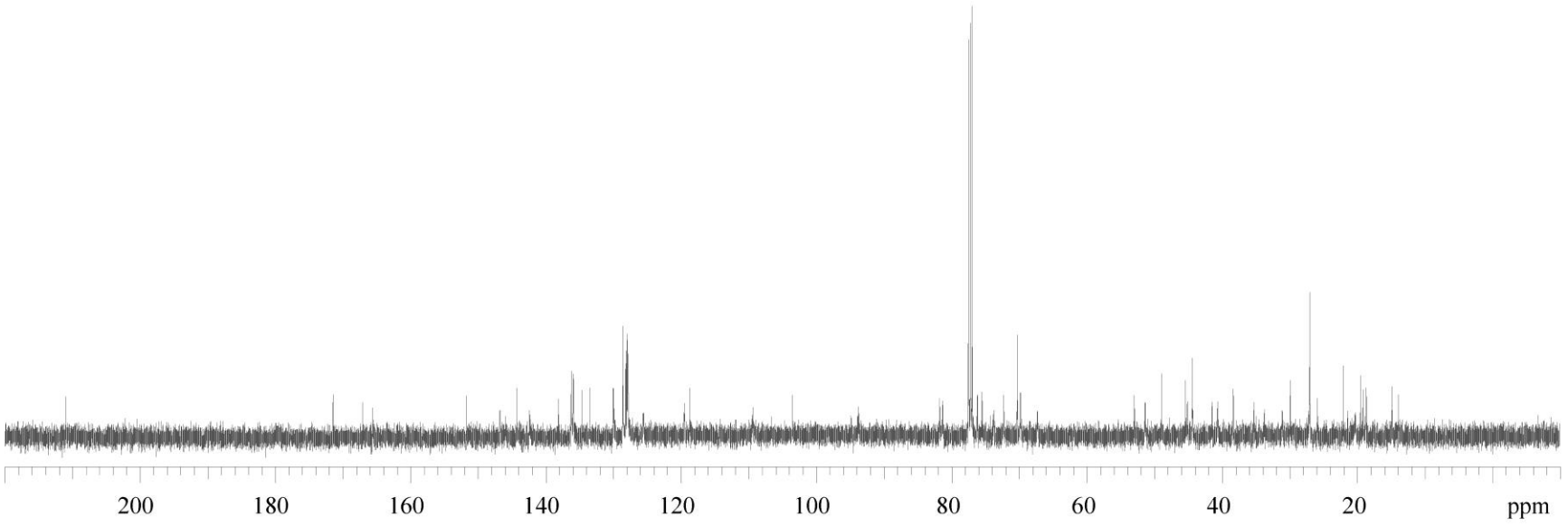

Me
Me
OTES
O
O
O
OMe
H
OBPS
O
Me
Me
Me
O
Me
OBOM
O
O
CO2Me

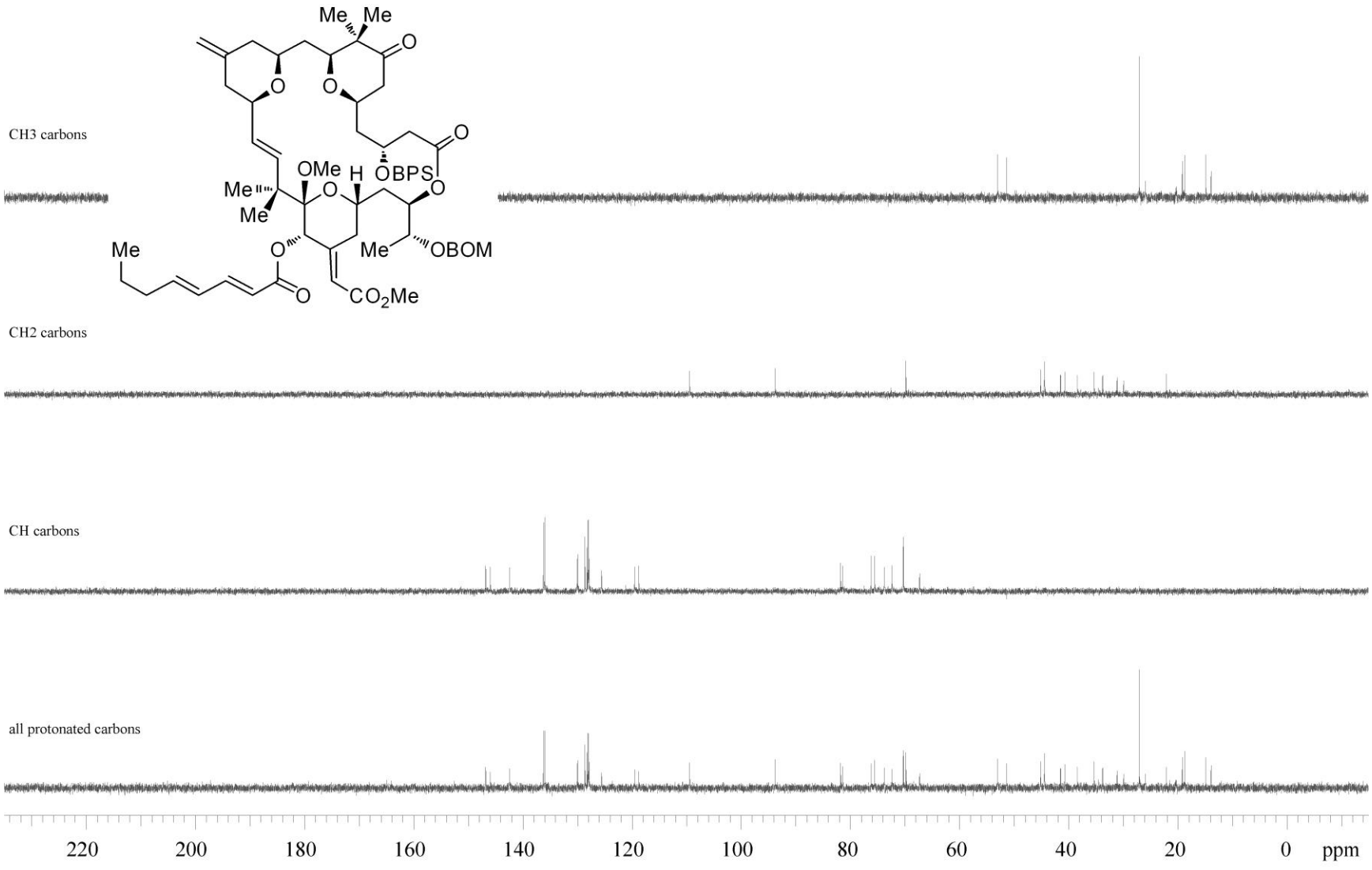
200
180
160
140
120
100
80
60
40
20
ppm

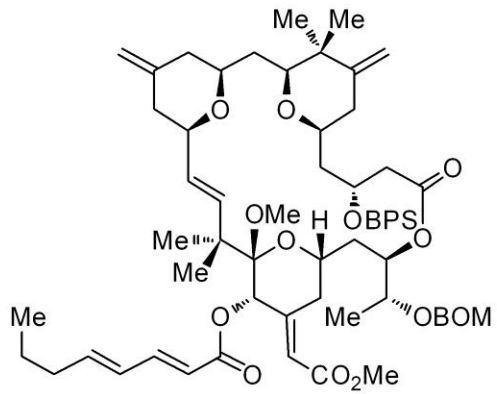

Me Me
OTES
O
O
O
OMe H OBPS
O
Me
Me
Me
O
Me
OBOM
O
O
CO2Me
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm


Me
Me
OMe
H
OBPS
OBOM
Me
CO_2Me
Me
Me
Me
ppm
10
9
8
7
6
5
4
3
2
1
-0

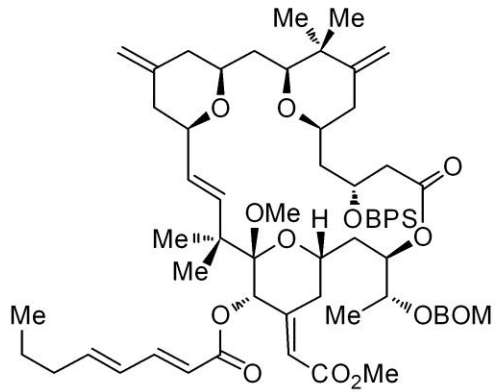

Me
Me
O
O
O
O
OMe
H
OBPS
O
Me
Me
Me
O
Me
OBOM
O
CO2Me


200
180
160
140
120
100
80
60
40
20
ppm

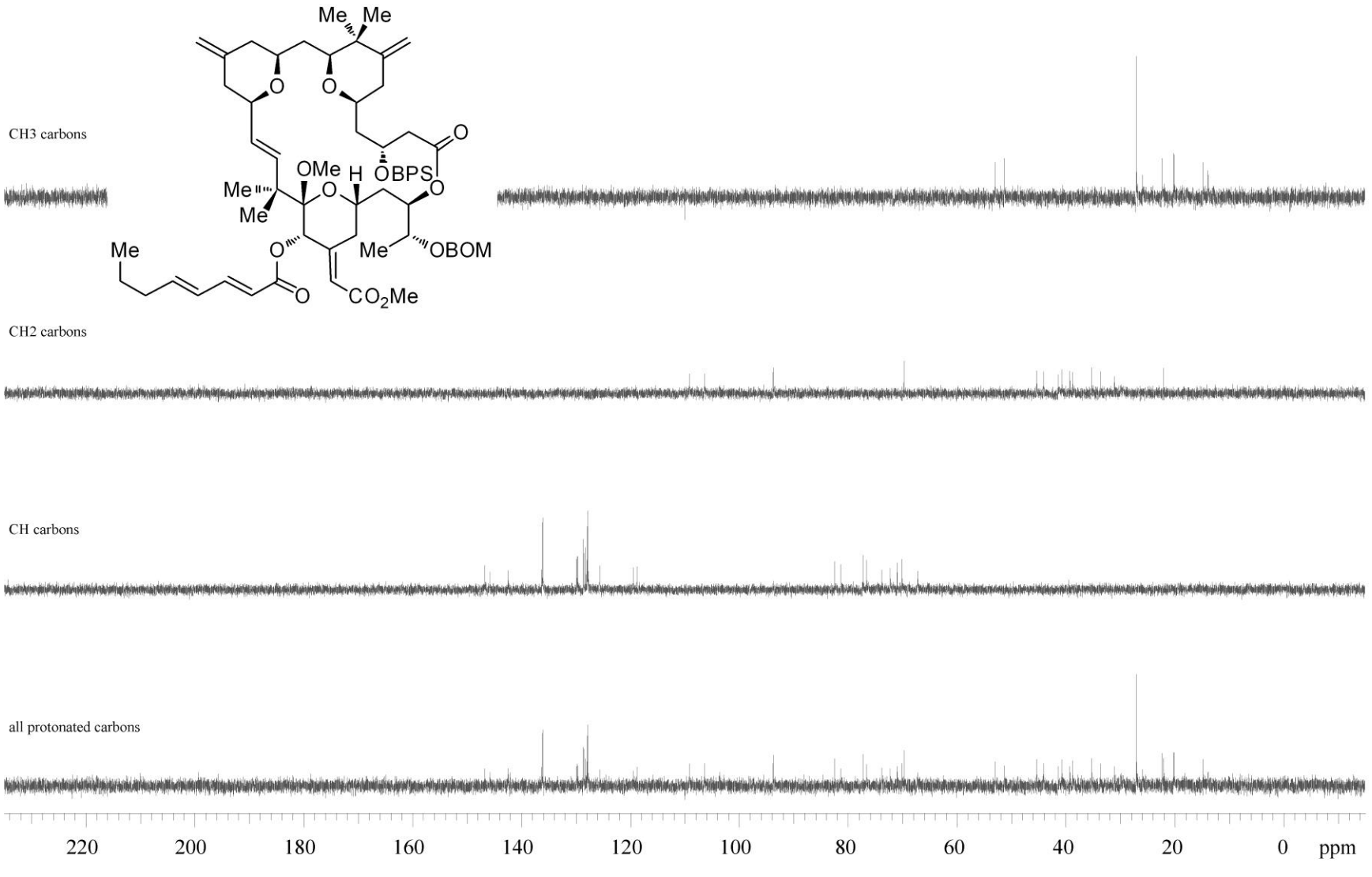

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
Me
Me
O
O
O
O
OMe
H
OBPS
O
Me
Me
Me
O
Me
OBOM
O
CO2Me
220
200
180
160
140
120
100
80
60
40
20
0
ppm

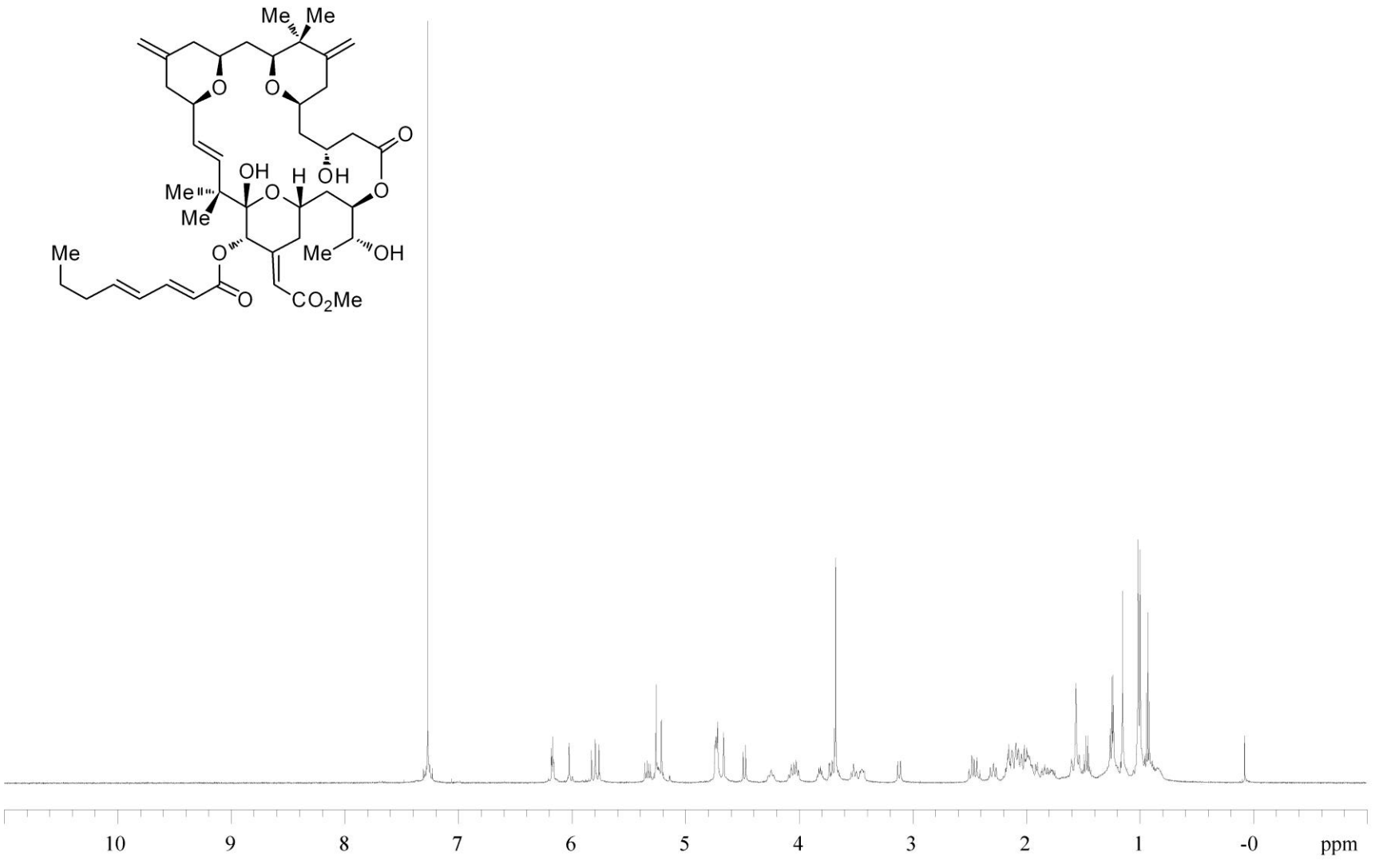

Me
Me
O
O
O
OMe
H
OBPS
O
Me
Me
O
Me
OBOM
Me
O
O
CO2Me

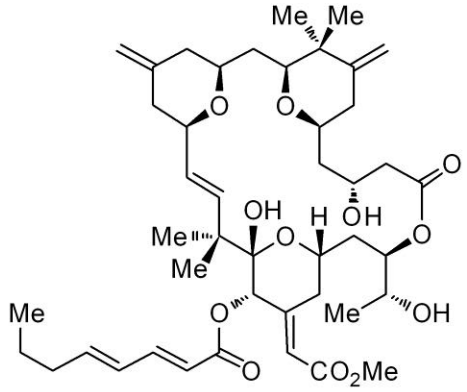
10
9
8
7
6
5
4
3
2
1
-0
ppm


Me
Me
O
O
O
OMe
H
OBPS
O
Me
Me
O
Me
OBOM
Me
O
O
CO2Me

200
180
160
140
120
100
80
60
40
20
ppm


CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
Me
Me
O
O
O
OMe
H
OBPS
O
Me
Me
Me
O
Me
OBOM
O
CO2Me
220
200
180
160
140
120
100
80
60
40
20
0
ppm

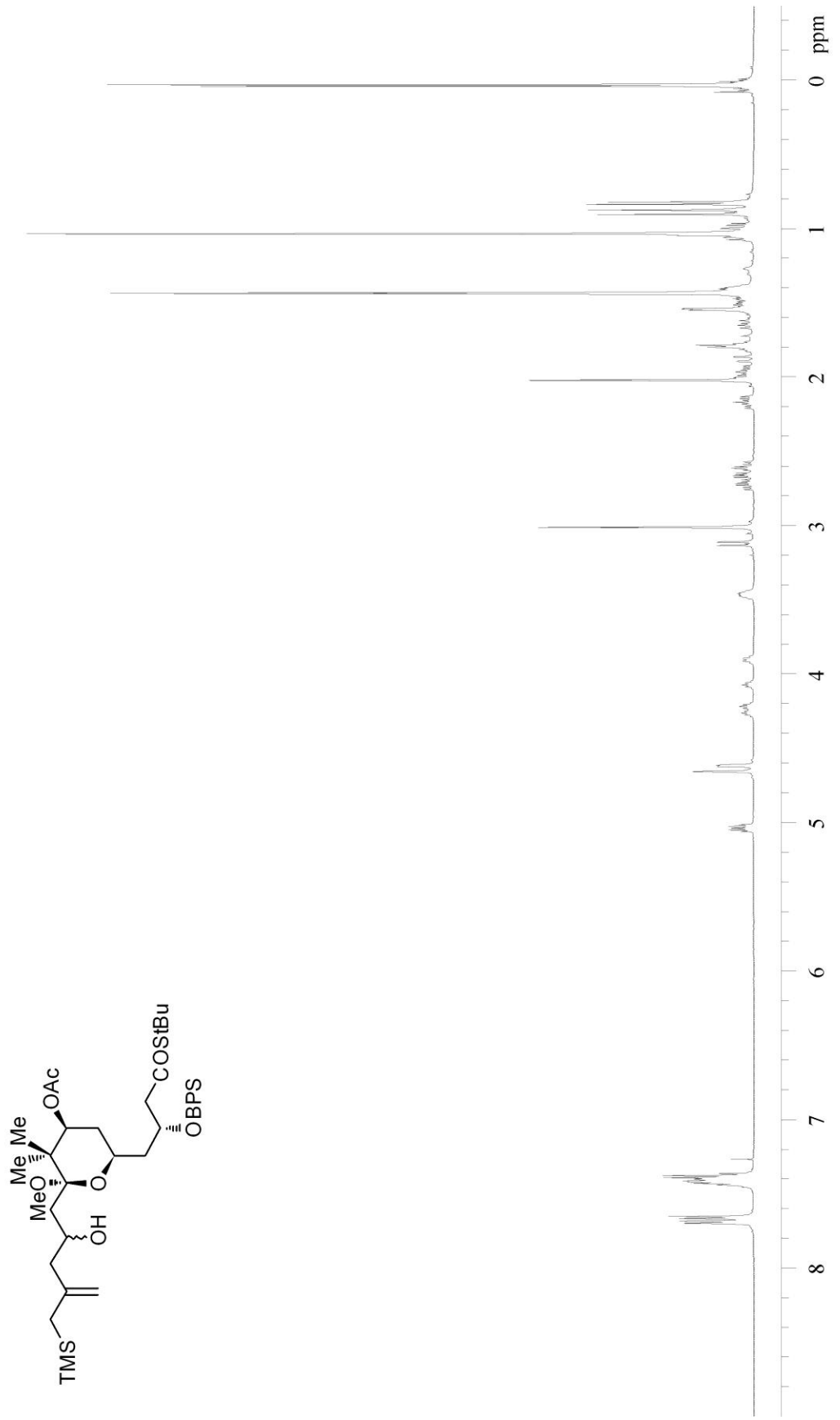

Me
Me
O
O
O
OH
H
OH
O
Me
Me
O
Me
OH
O
O
CO2Me
10
9
8
7
6
5
4
3
2
1
-0
ppm

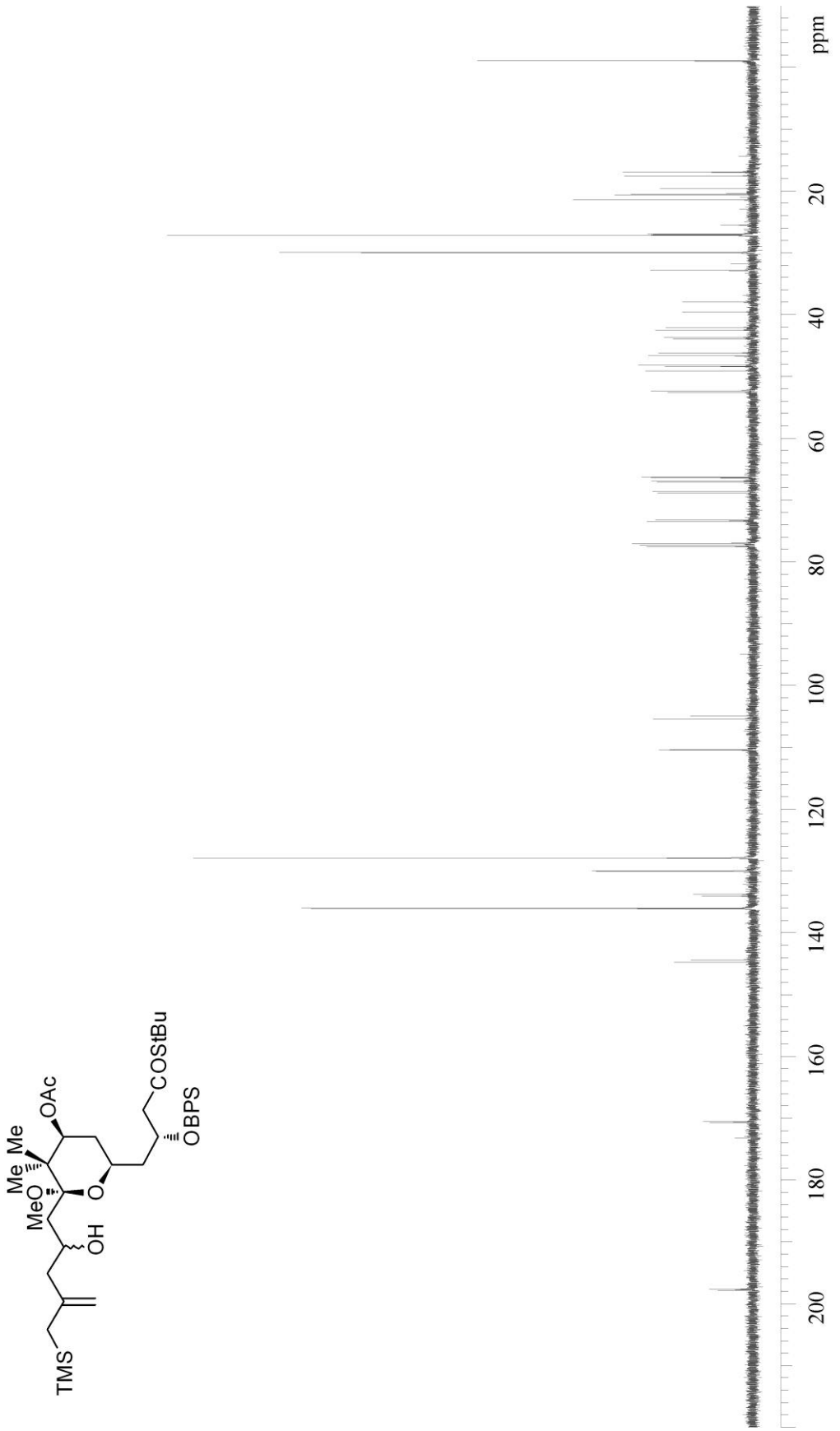

Me
Me
O
O
O
OH
H
OH
O
Me
Me
Me
O
Me
OH
O
CO2Me

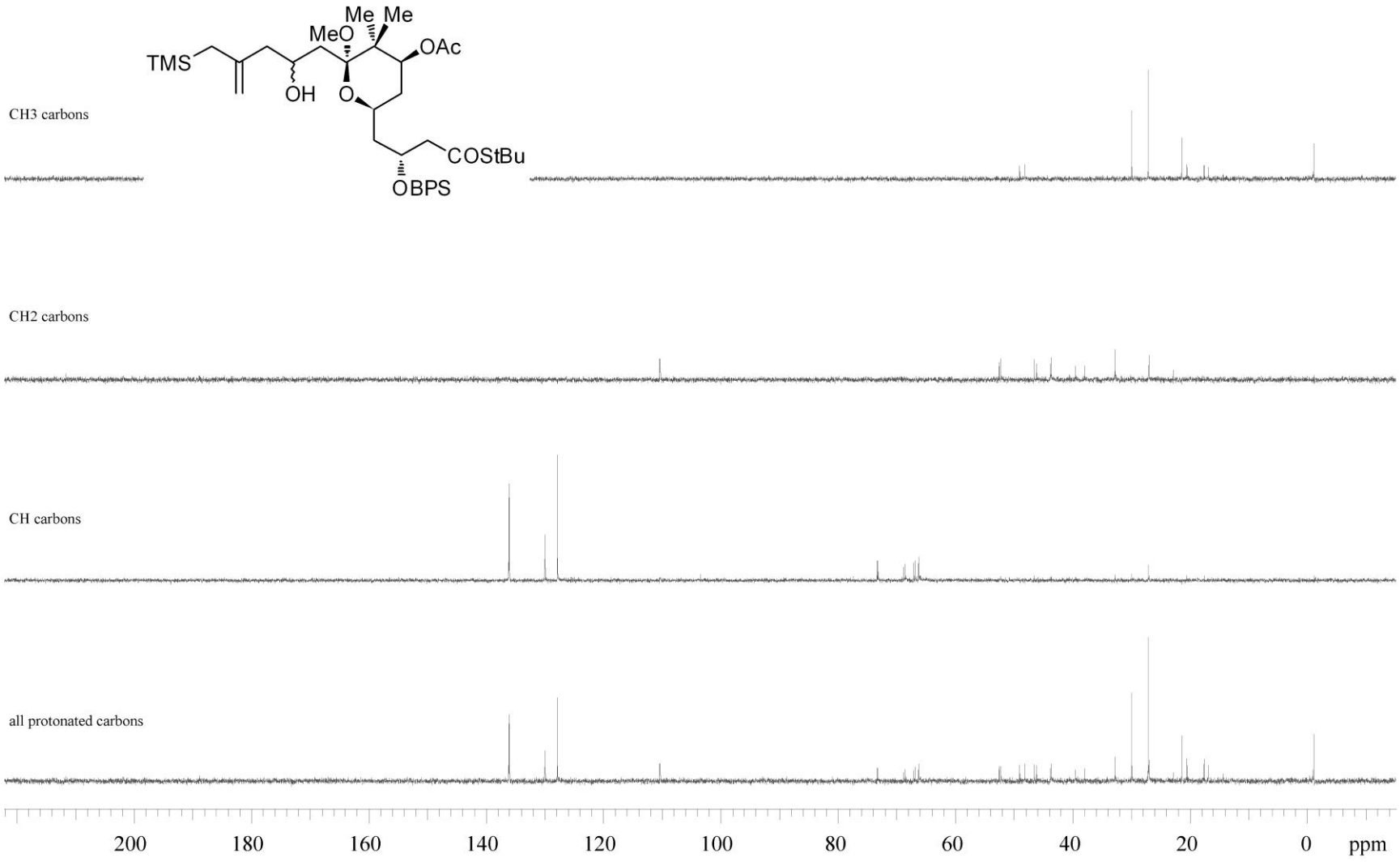
200
180
160
140
120
100
80
60
40
20
ppm

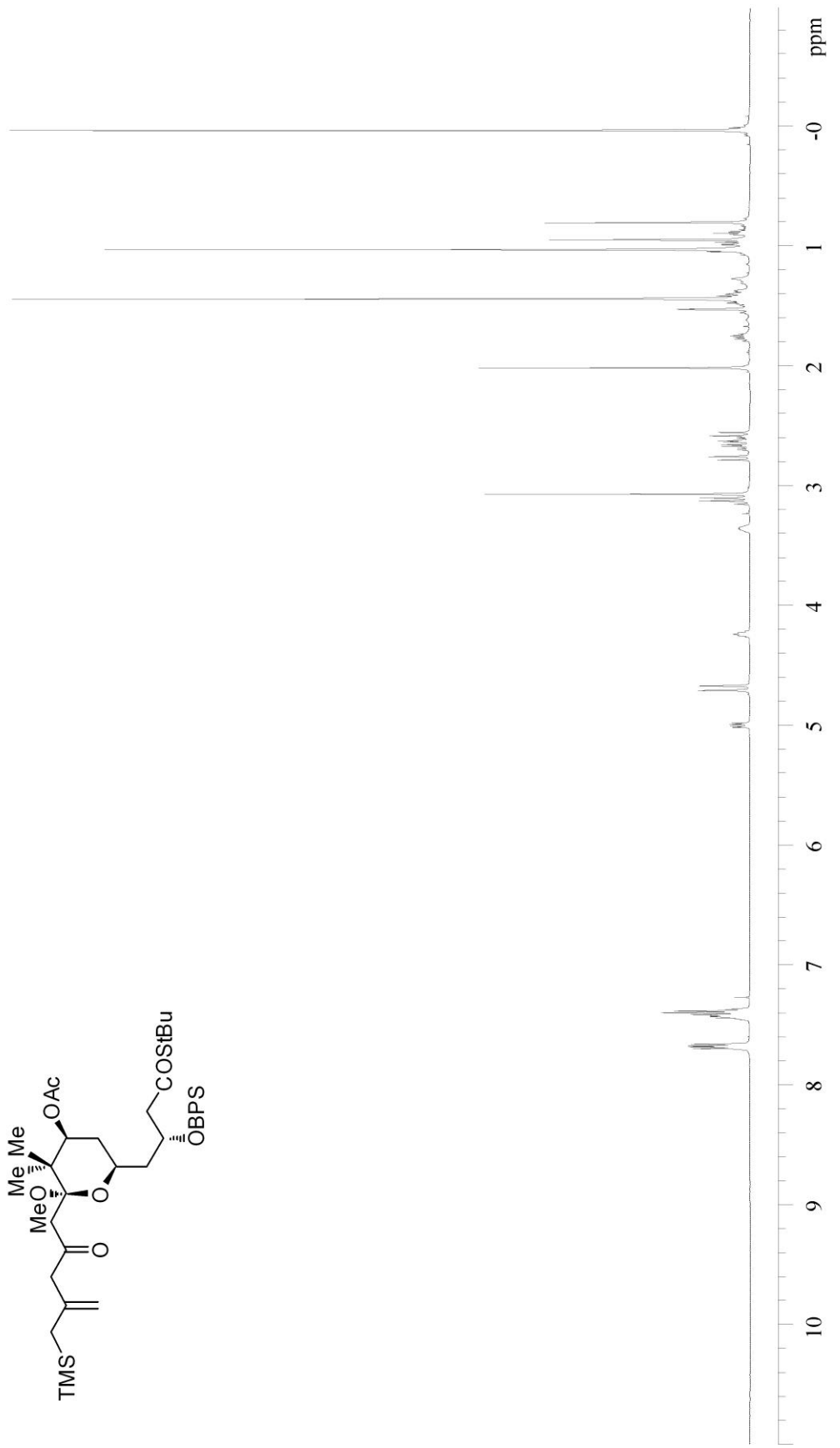
APPENDIX B

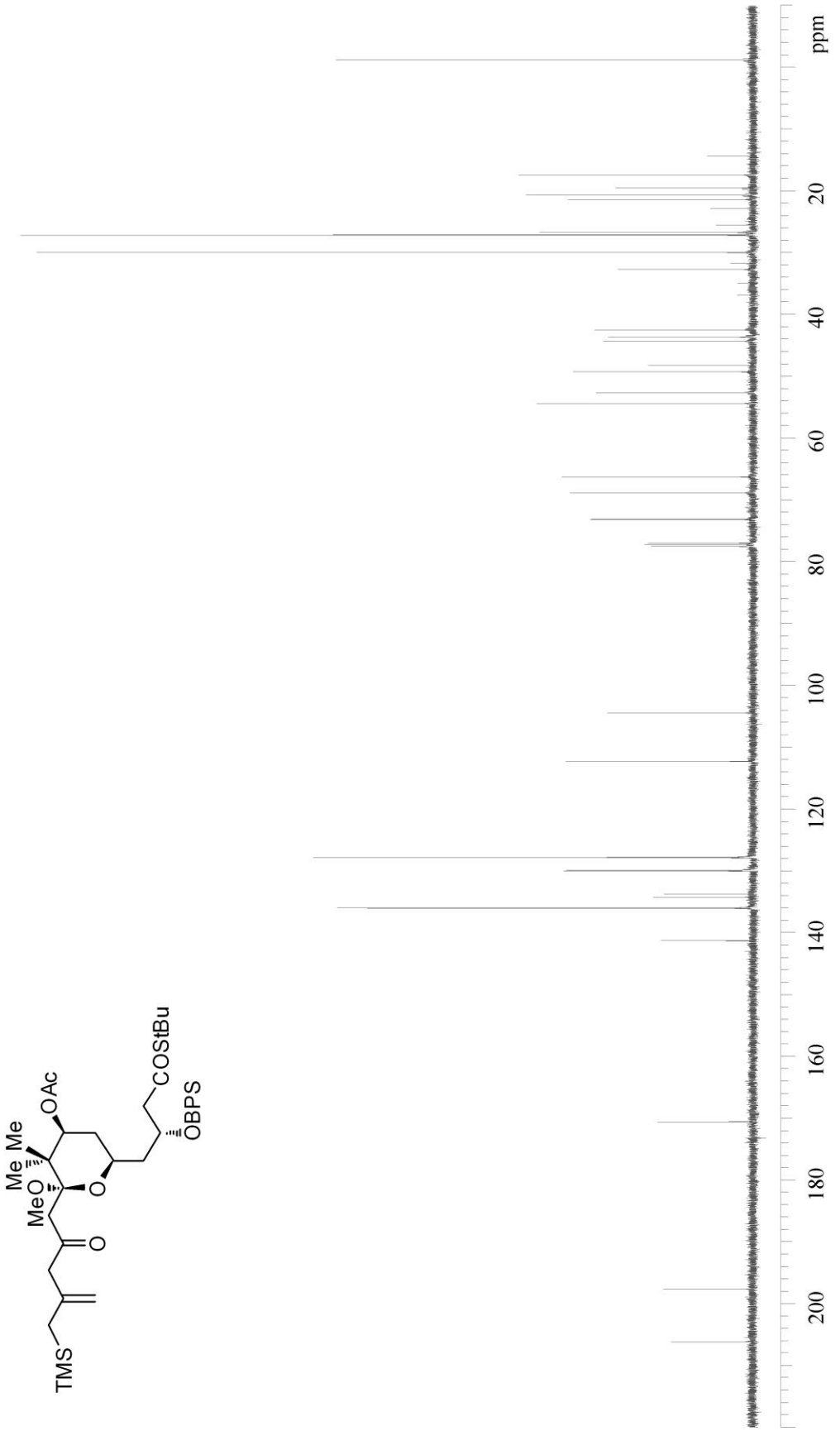
^{1}H, AND ^{13}C NMR SPECTRA FOR CHAPTER 2

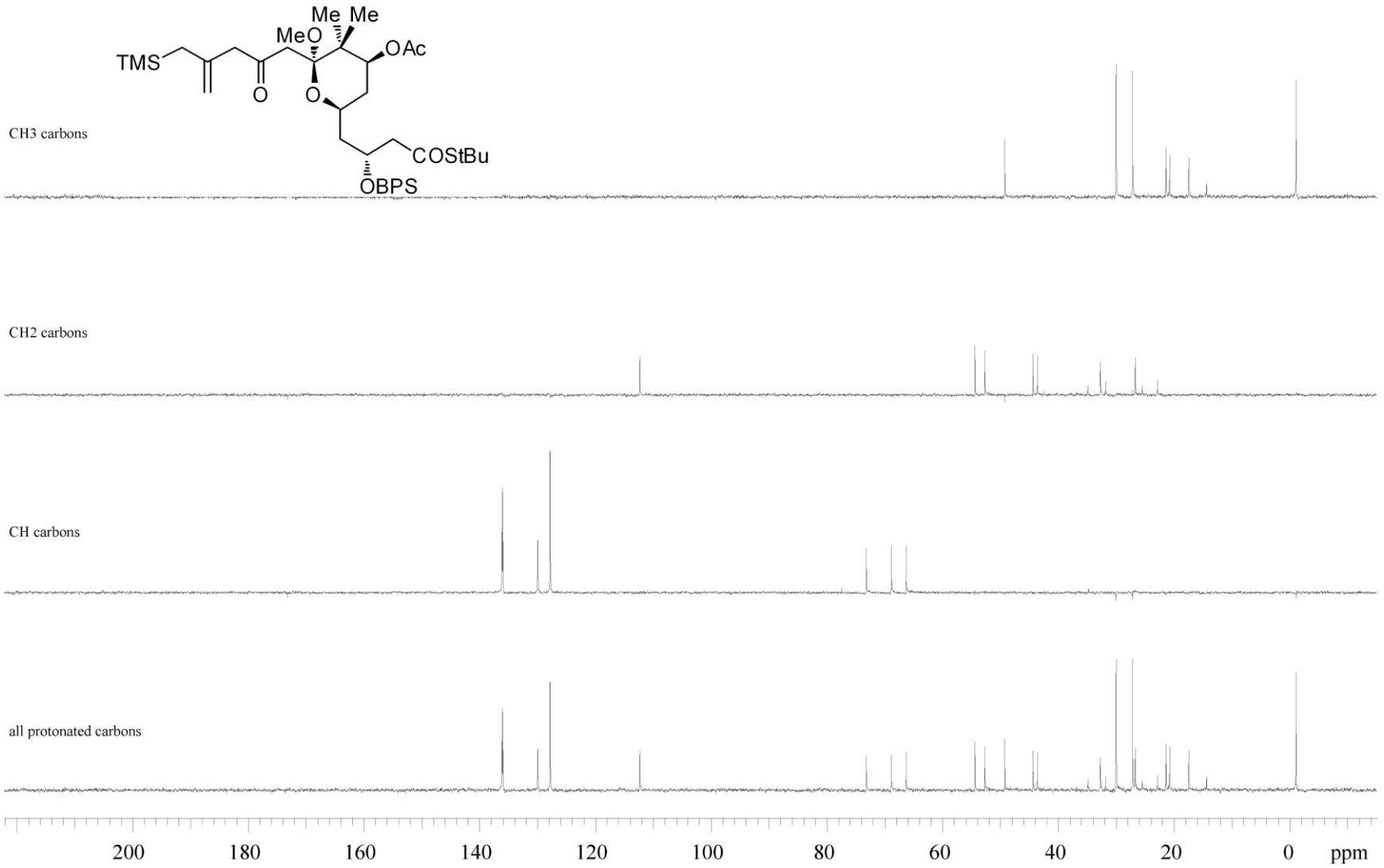

TMS
OH
MeO
Me Me
OAc
O
OBPS
COStBu
8
7
6
5
4
3
2
1
0
ppm

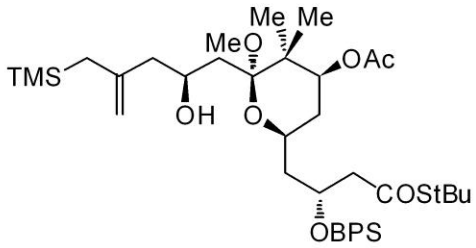

TMS
OH
MeO
Me Me
OAc
O
OBPS
COStBu
200
180
160
140
120
100
80
60
40
20
ppm


Me Me
MeO
OAc
TMS
OH
O
COStBu
OBPS
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

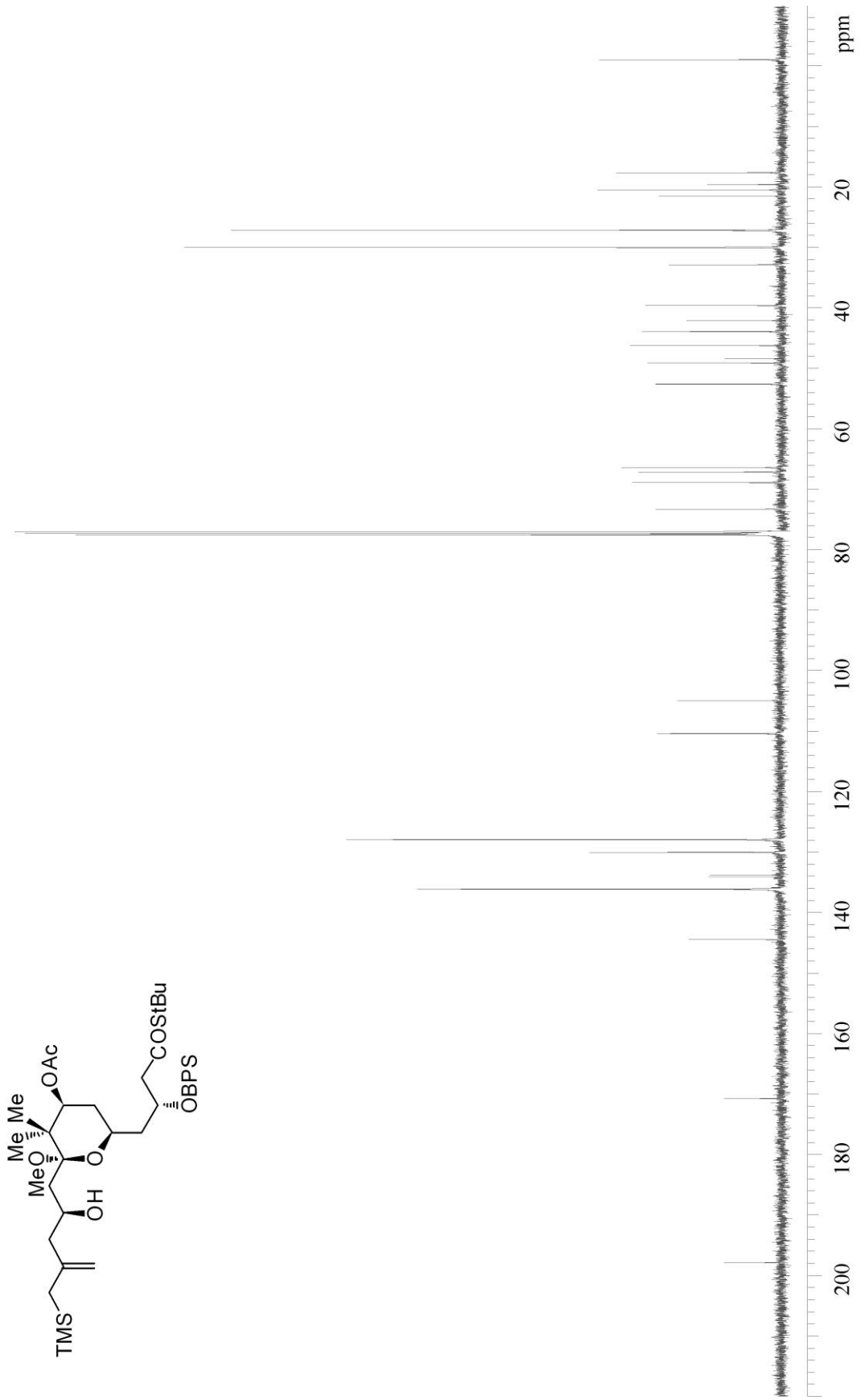

Me Me
MeO
OAc
O
O
COStBu
OBPS
TMS
10
9
8
7
6
5
4
3
2
1
-0
ppm

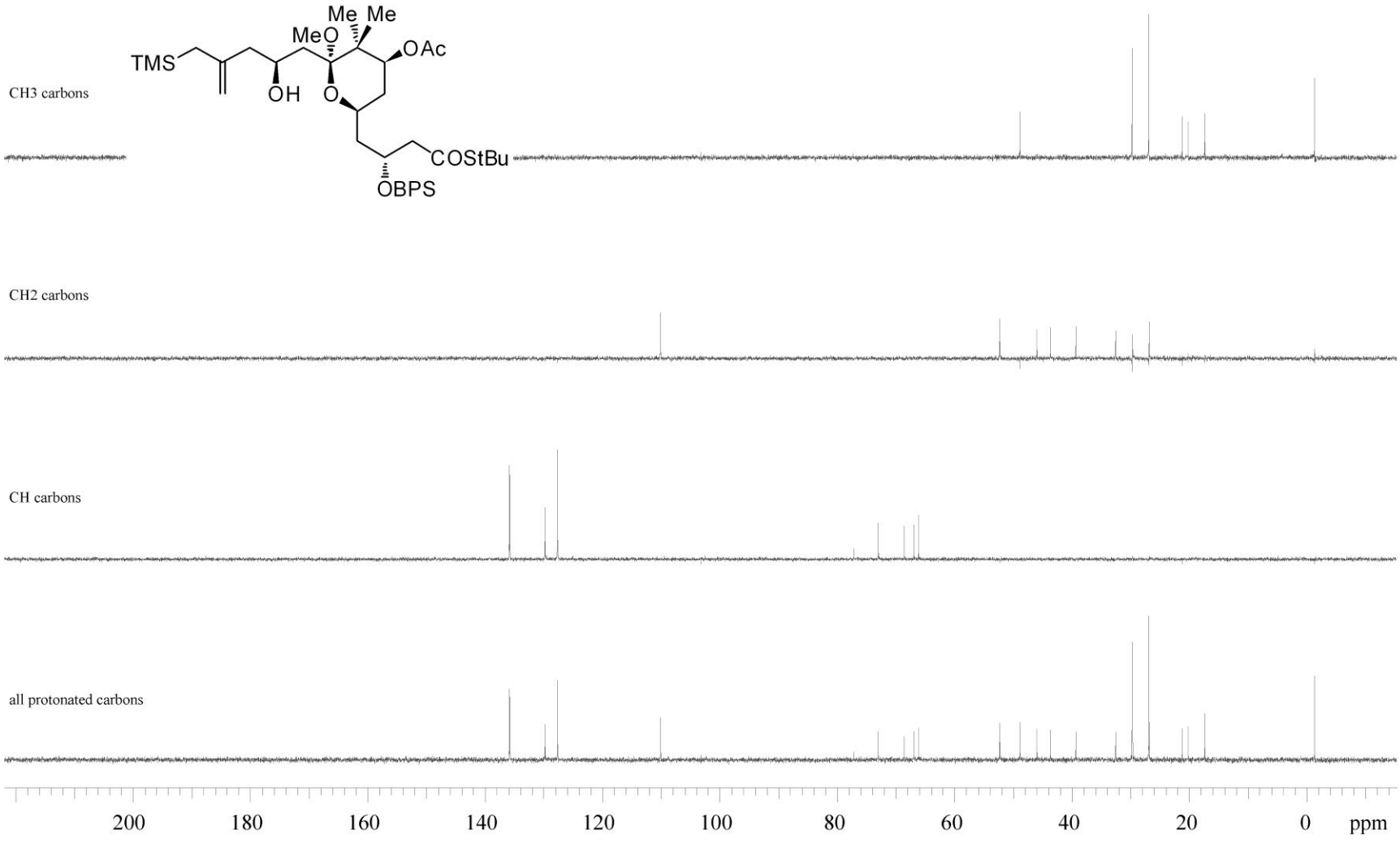

TMS
O
MeO
Me Me
OAc
O
COStBu
OBPS
ppm
200
180
160
140
120
100
80
60
40
20

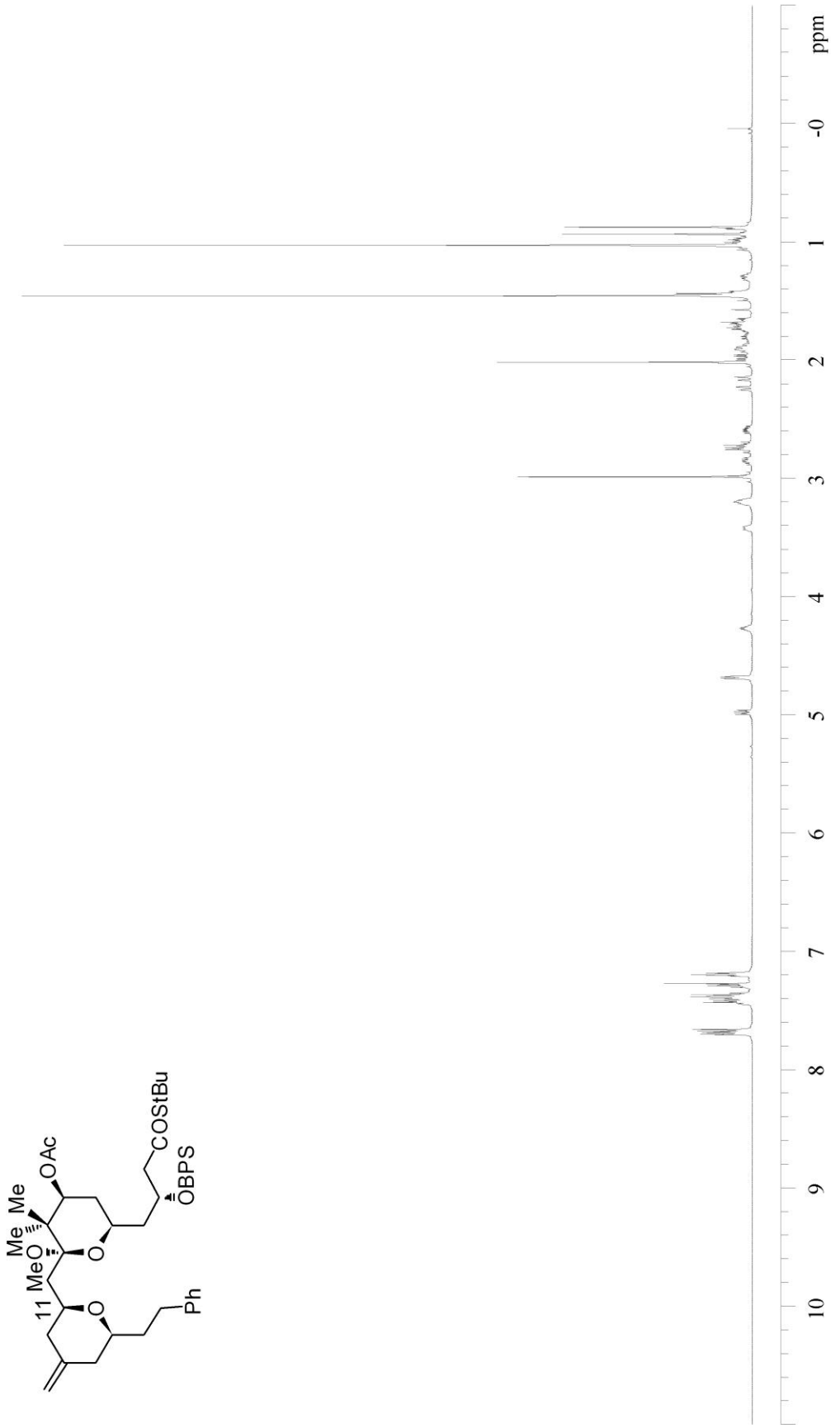

Me Me
MeO
OAc
TMS
O
O
COStBu
OBPS
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

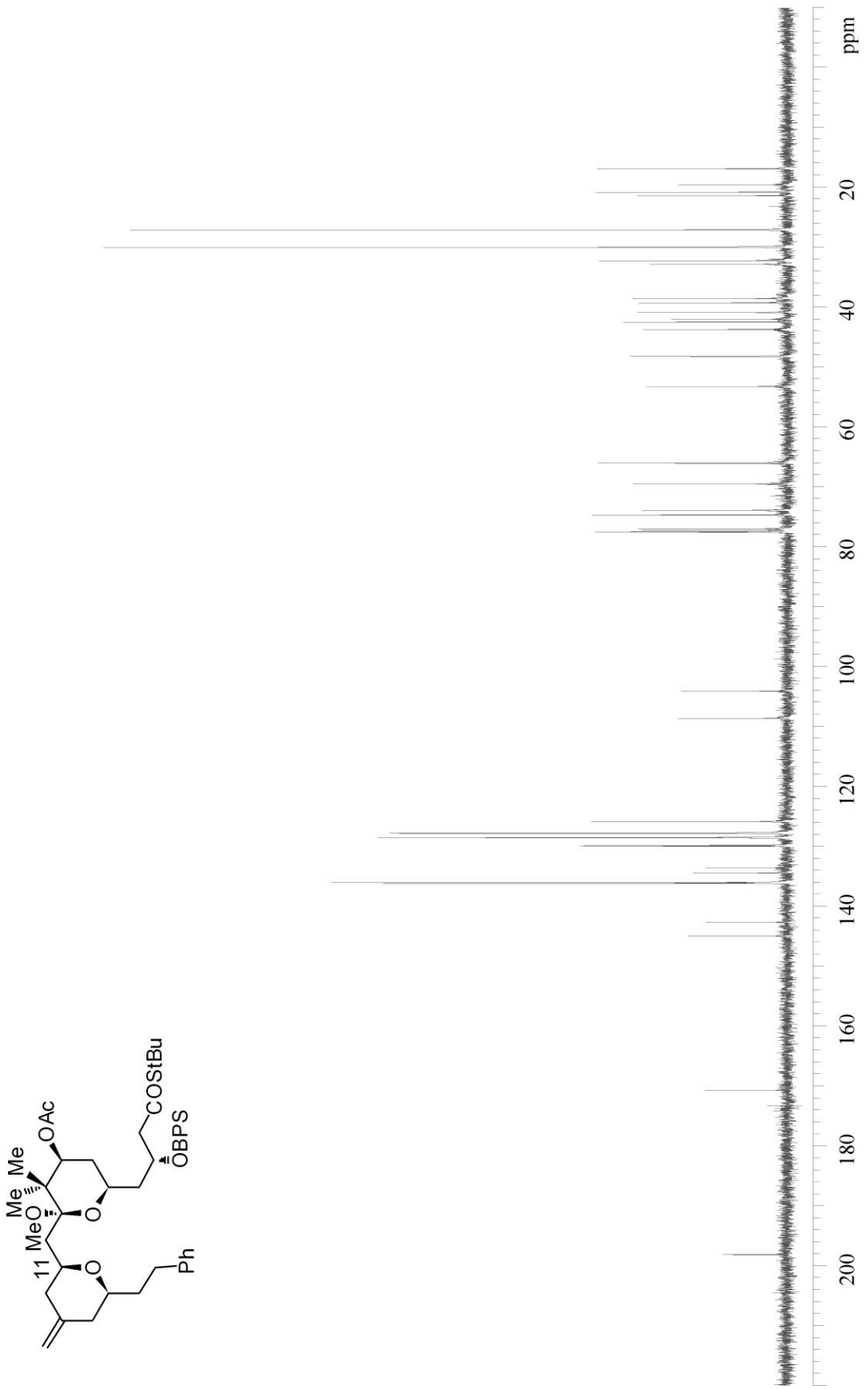

Me Me
MeO
OAc
TMS
OH
O
COStBu
OBPS

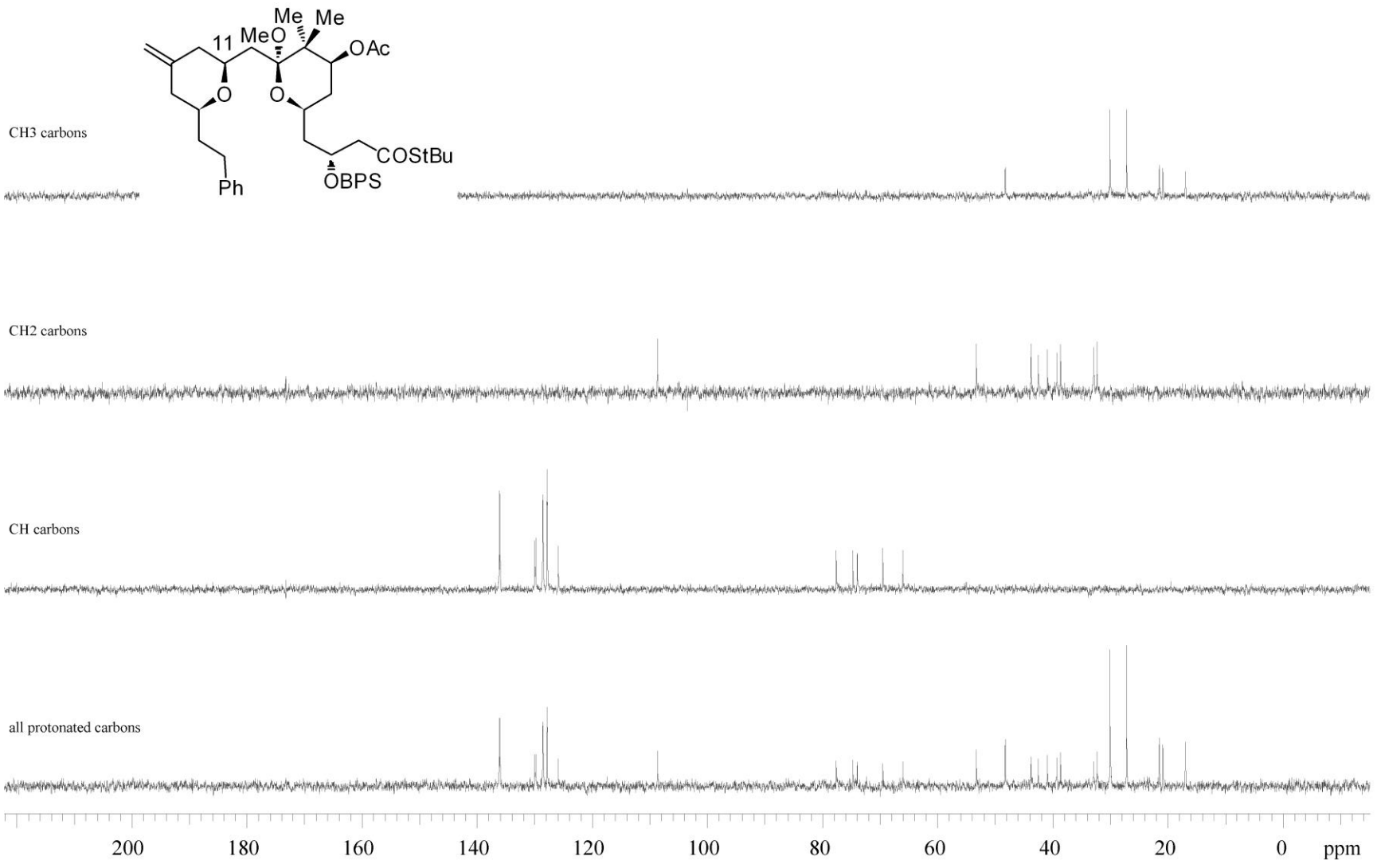
10
9
8
7
6
5
4
3
2
1
-0
ppm

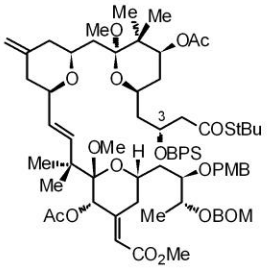

TMS
OH
MeO
Me Me
OAc
O
COStBu
OBPS
ppm
200
180
160
140
120
100
80
60
40
20


Me Me
MeO
OAc
TMS
OH
O
COStBu
OBPS
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

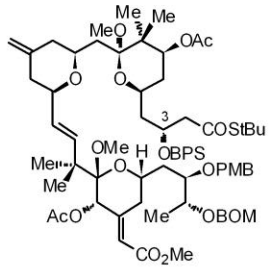

Me
Me
OAc
MeO
11
O
O
Ph
COStBu
OBPS
10
9
8
7
6
5
4
3
2
1
-0
ppm

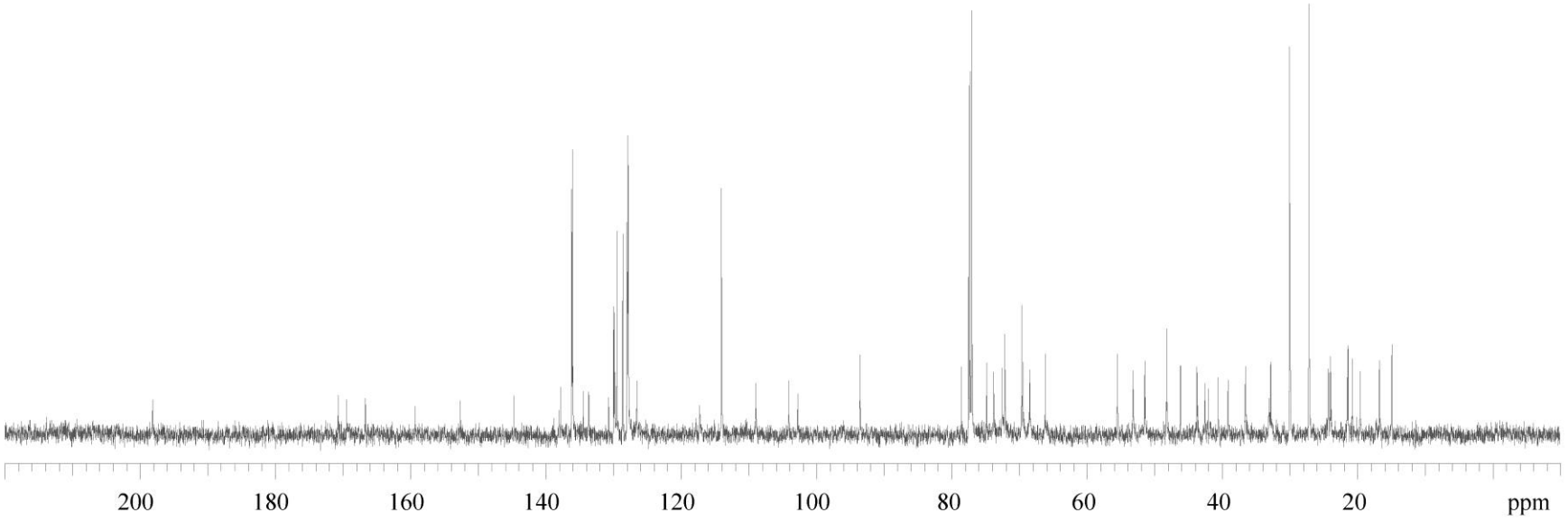

Me
Me
OAc
COStBu
OBPS
Me
MeO
11
O
O
Ph
ppm
20
40
60
80
100
120
140
160
180
200

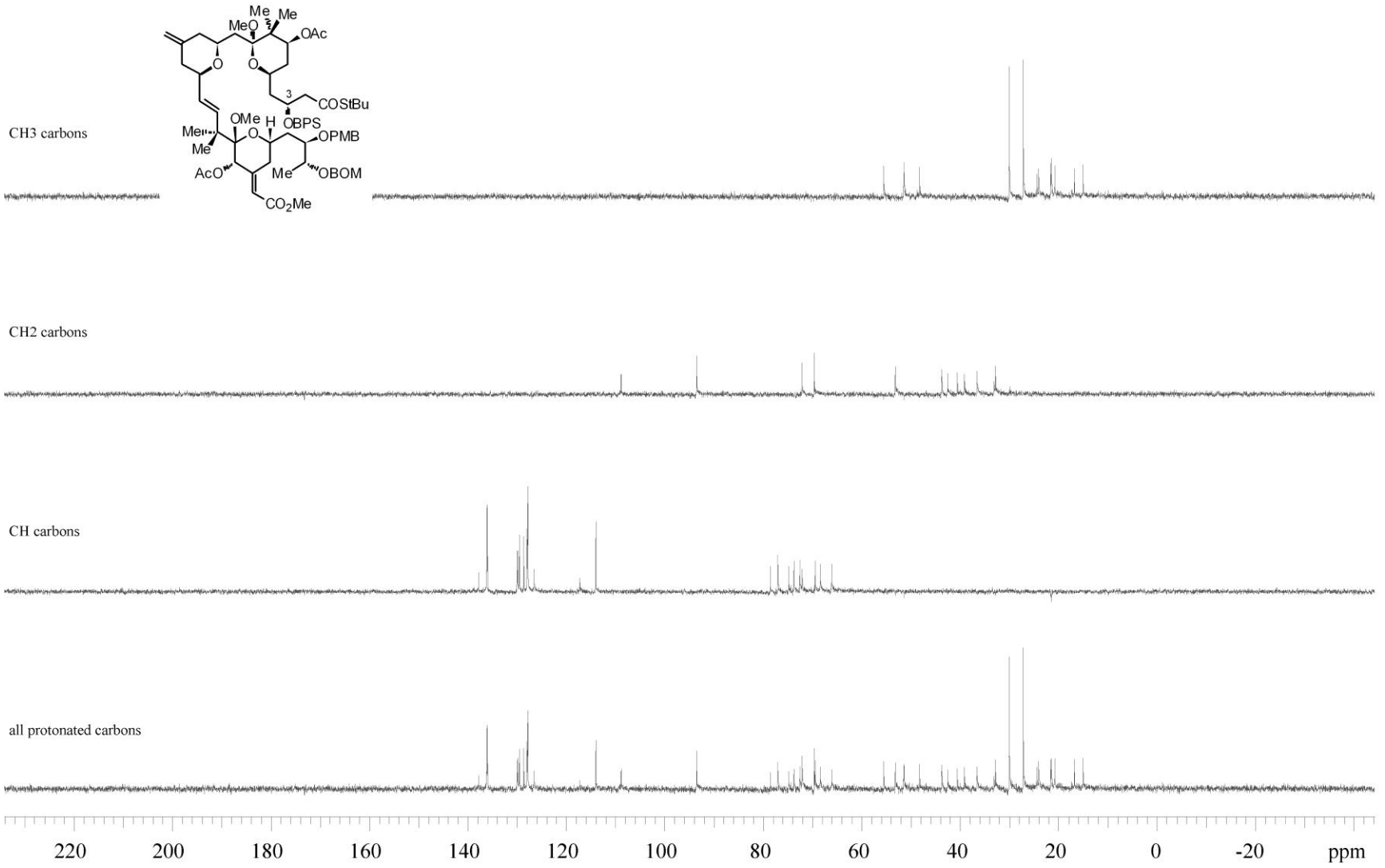

Me
Me
11
MeO
OAc
O
O
COStBu
OBPS
Ph
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
200
180
160
140
120
100
80
60
40
20
0
ppm

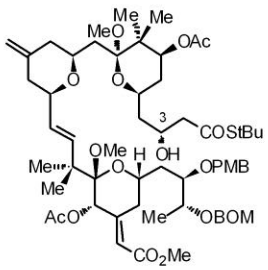

Me
Me
MeO
OAc
O
O
3
COStBu
OMe
H
OBPS
Me
Me
O
OPMB
AcO
Me
OBOM
CO2Me

10
9
8
7
6
5
4
3
2
1
-0
ppm

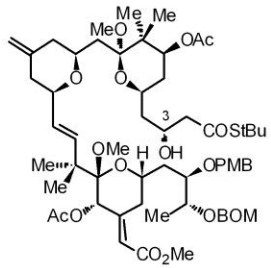

Me
Me
MeO
OAc
O
O
3
COStBu
OMe
H
OBPS
Me
Me
O
OPMB
AcO
Me
OBOM
CO2Me

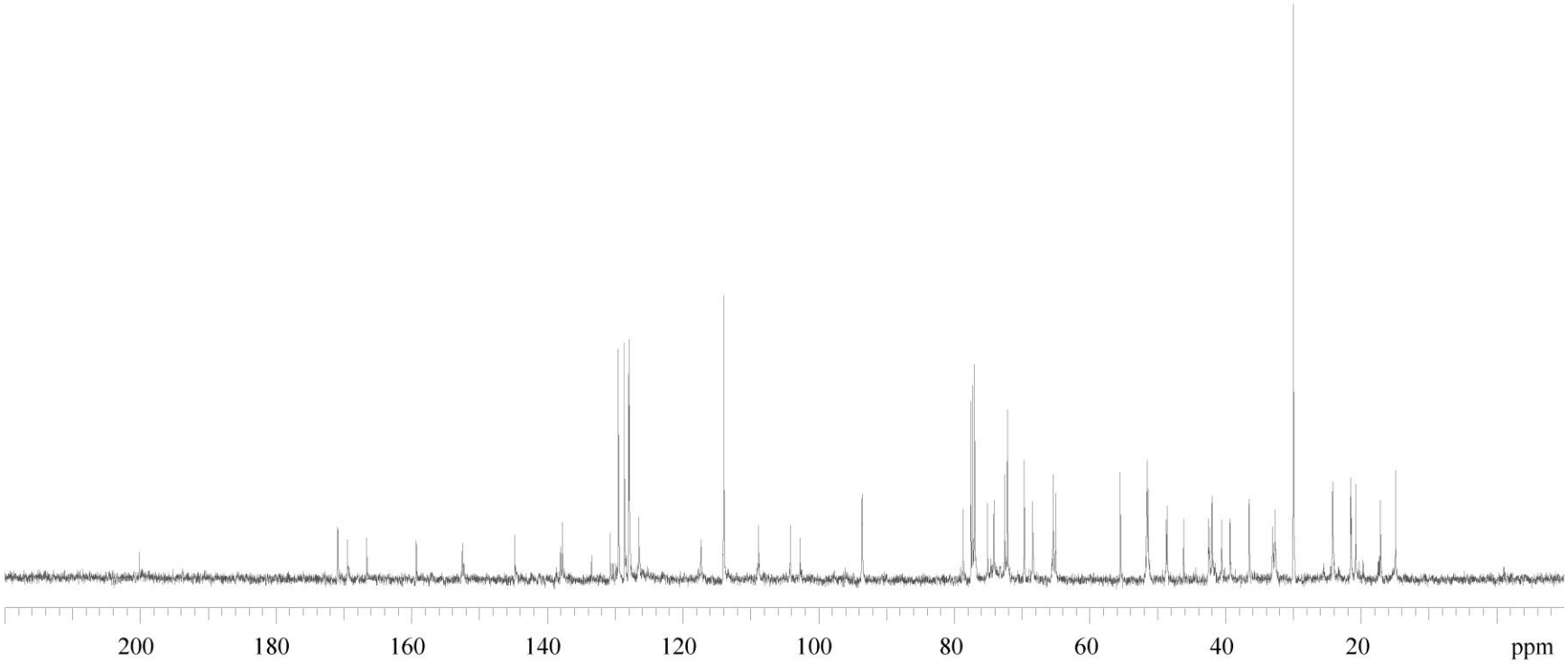

200
180
160
140
120
100
80
60
40
20
ppm

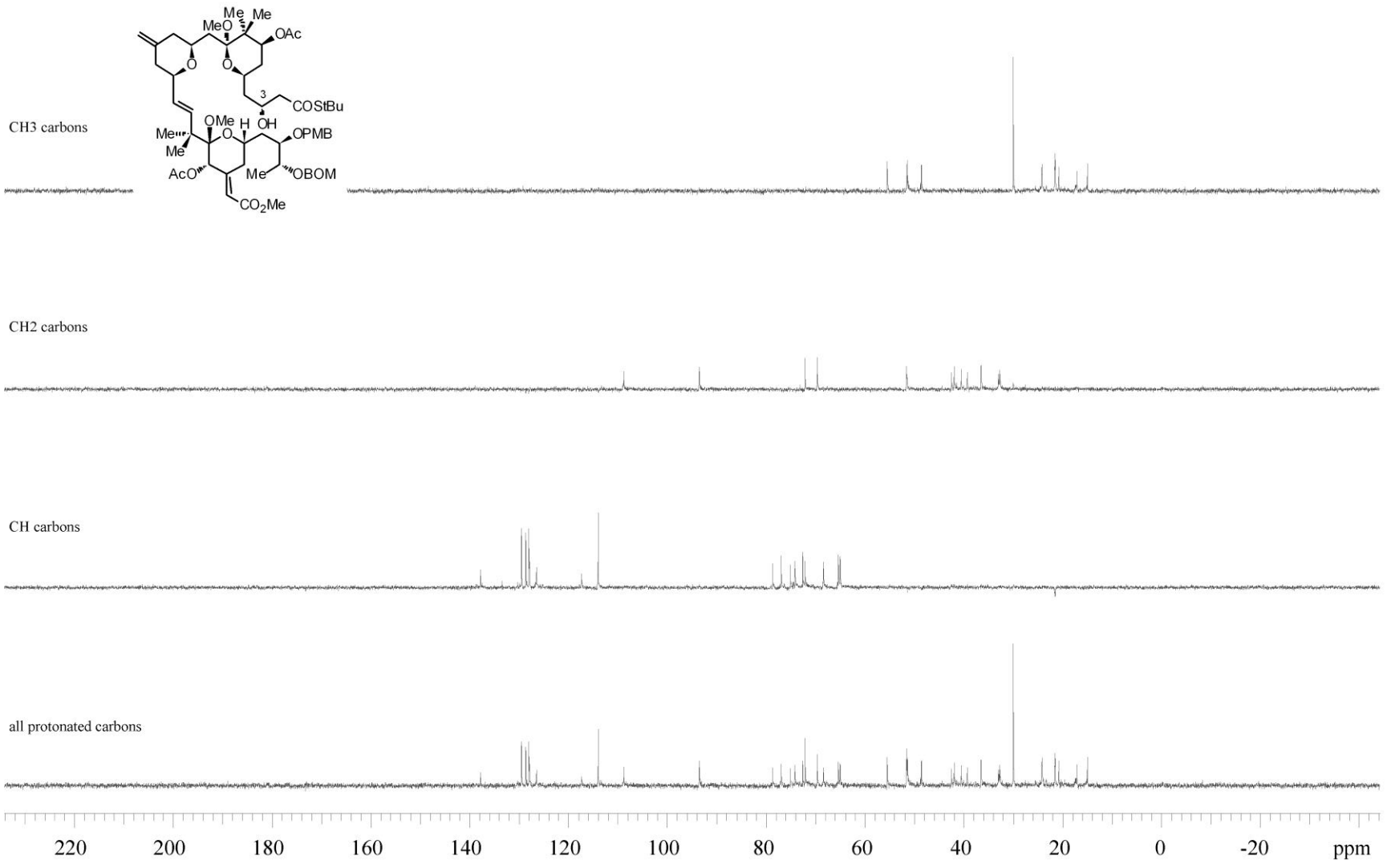

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
Me
Me
MeO
OAc
O
O
3
COStBu
OMe
H
OBPS
Me
Me
O
OPMB
AcO
Me
OBOM
CO2Me
220
200
180
160
140
120
100
80
60
40
20
0
-20
ppm

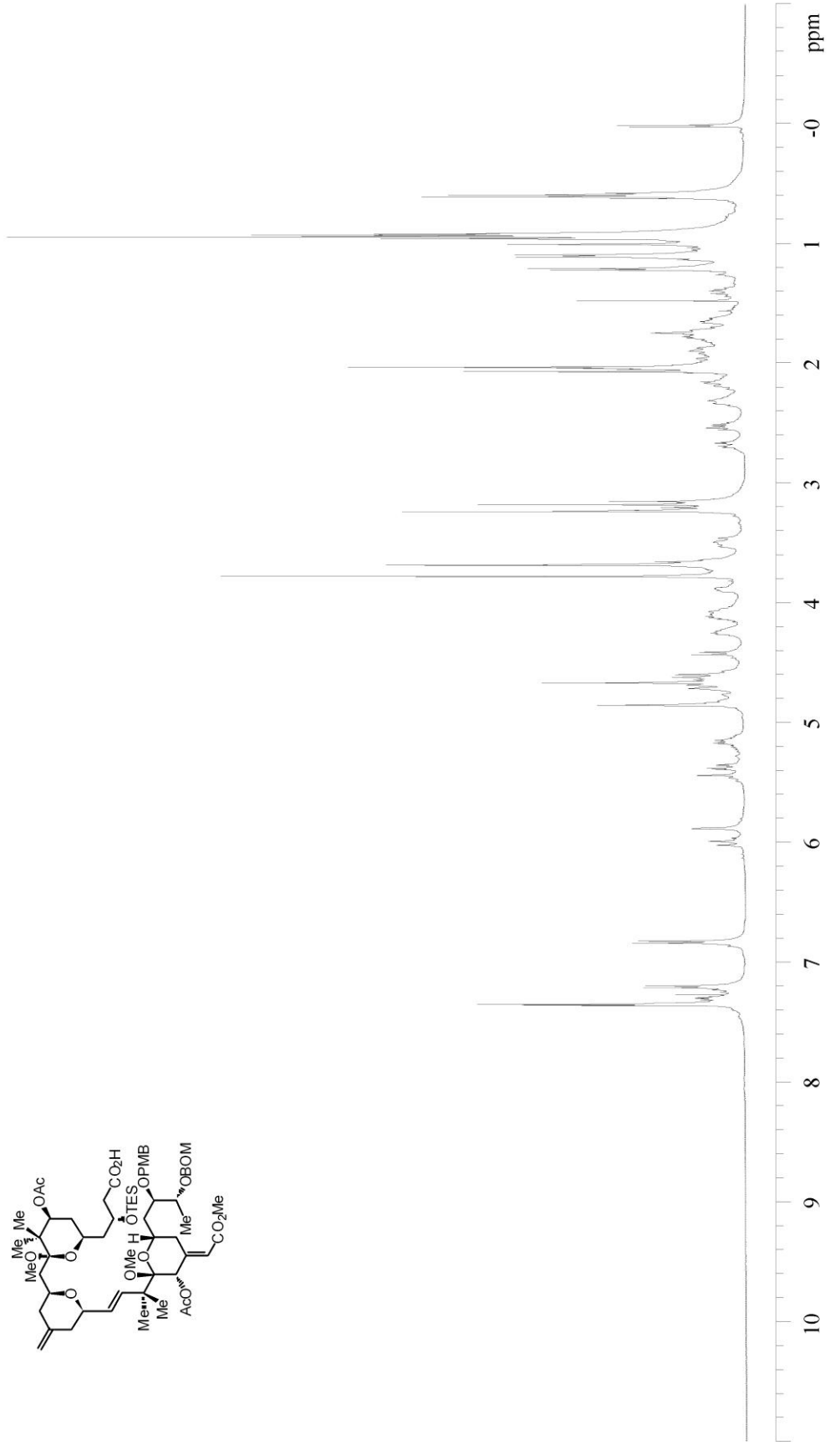

Me
Me
MeO
OAc
O
O
3
COStBu
OMe
H
OH
OPMB
Me
Me
AcO
Me
OBOM
CO2Me

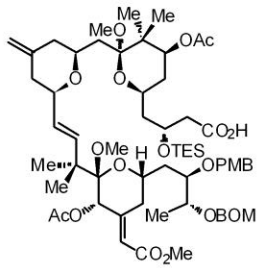
10
9
8
7
6
5
4
3
2
1
-0
ppm

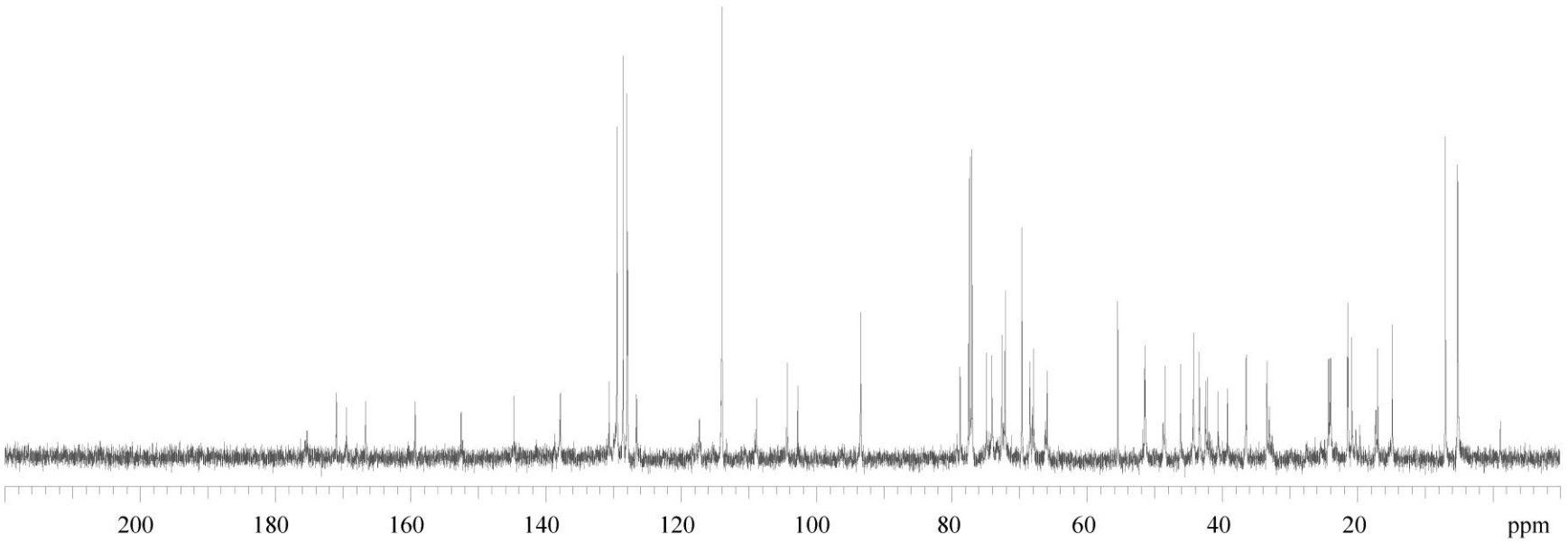

Me Me
MeO
OAc
O
O
3
COStBu
OMe H OH
OPMB
Me
Me
AcO
Me
OBOM
CO2Me

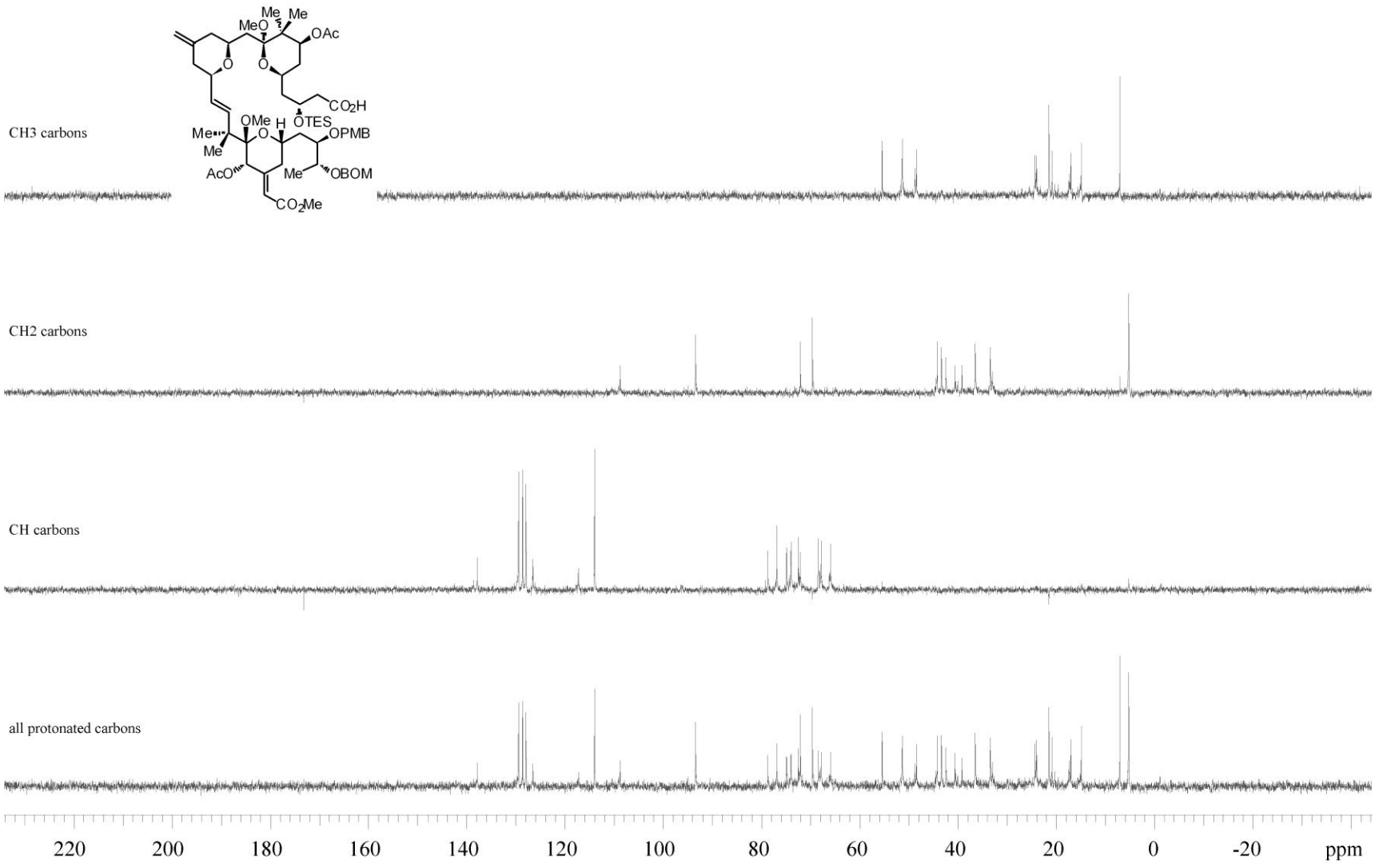

200
180
160
140
120
100
80
60
40
20
ppm

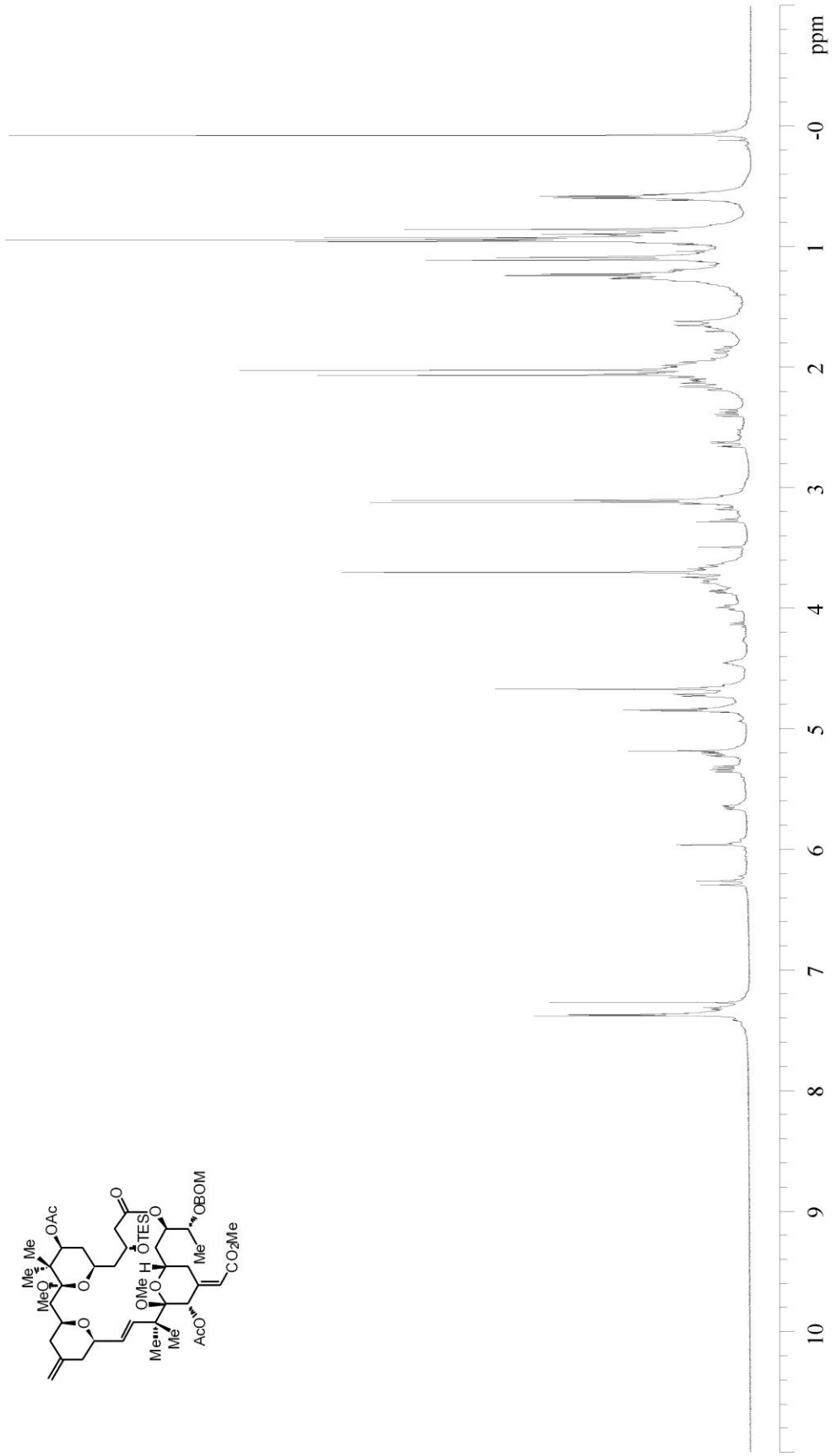

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
Me
Me
MeO
OAc
O
O
3
COStBu
OMe
H
OH
Me
Me
OPMB
AcO
Me
OBOM
CO2Me
220
200
180
160
140
120
100
80
60
40
20
0
-20
ppm

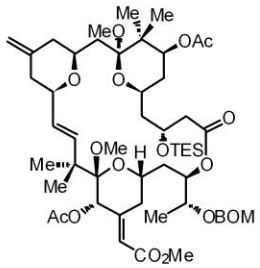

OAc
Me
Me
MeO
CO2H
OTES
OPMB
OMe
H
O
Me
Me
AcO
Me
OBOM
CO2Me
10
9
8
7
6
5
4
3
2
1
-0
ppm

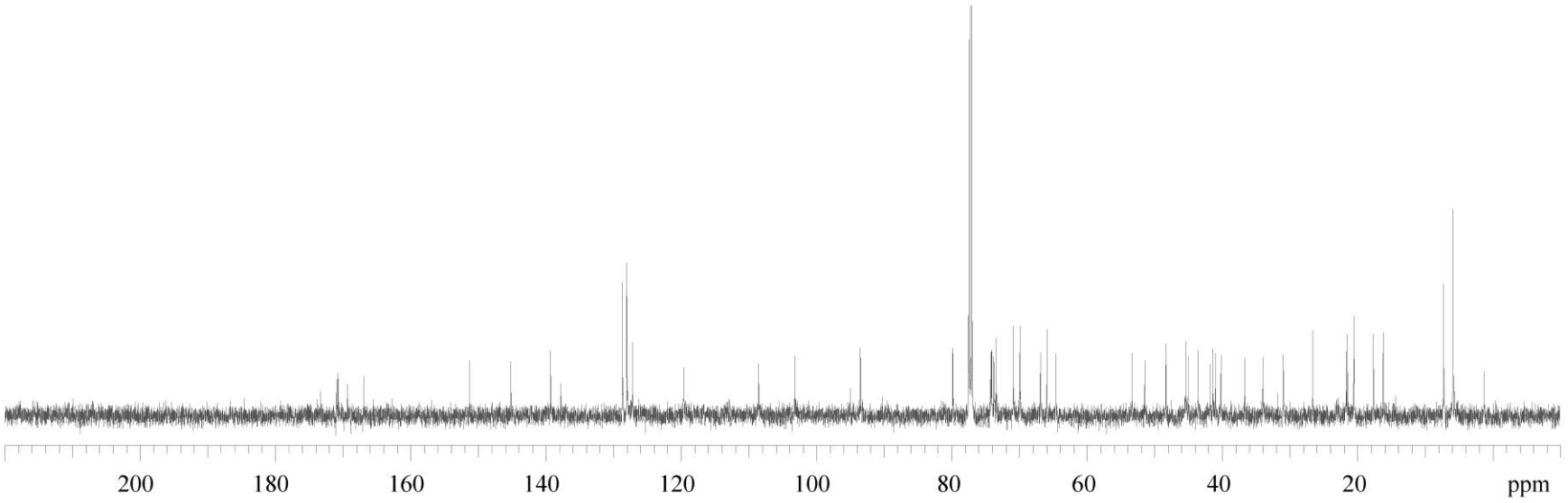

Me
Me
MeO
OAc
O
O
CO2H
OMe
H
OTES
OPMB
Me
Me
O
AcO
Me
OBOM
CO2Me

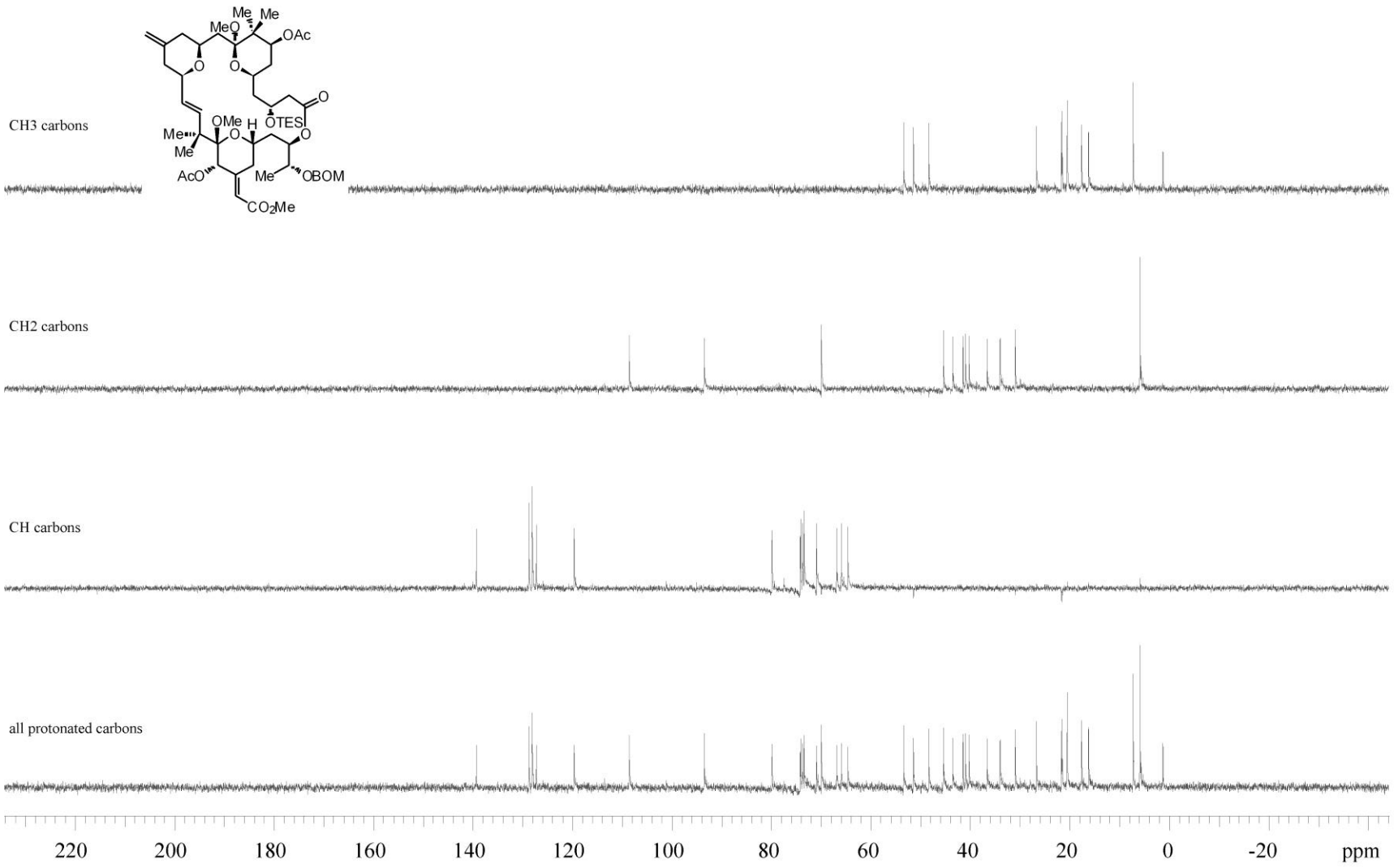

200
180
160
140
120
100
80
60
40
20
ppm

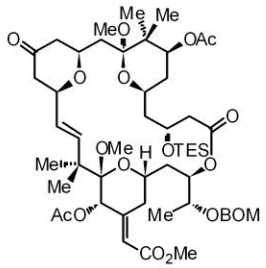

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
Me
Me
MeO
OAc
O
O
CO2H
OMe
H
OTES
Me
Me
OPMB
AcO
Me
OBOM
CO2Me
220
200
180
160
140
120
100
80
60
40
20
0
-20
ppm


Me
Me
MeO
OAc
O
O
O
OMe
H
OTES
O
Me
OBOM
Me
AcO
CO2Me
10
9
8
7
6
5
4
3
2
1
-0
ppm

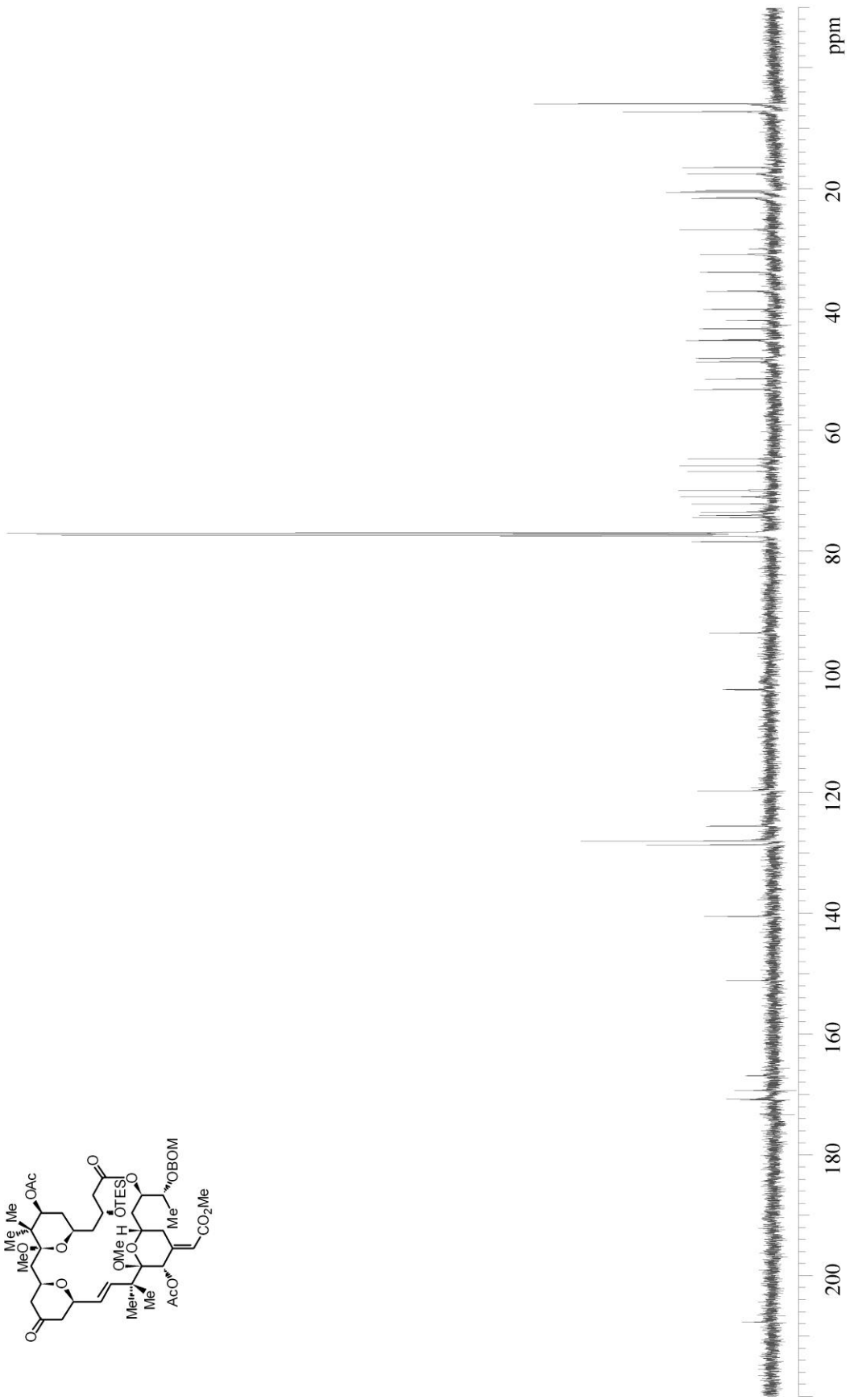

Me
Me
MeO
OAc
O
O
O
OMe
H
OTES
O
Me
Me
AcO
Me
OBOM
CO2Me

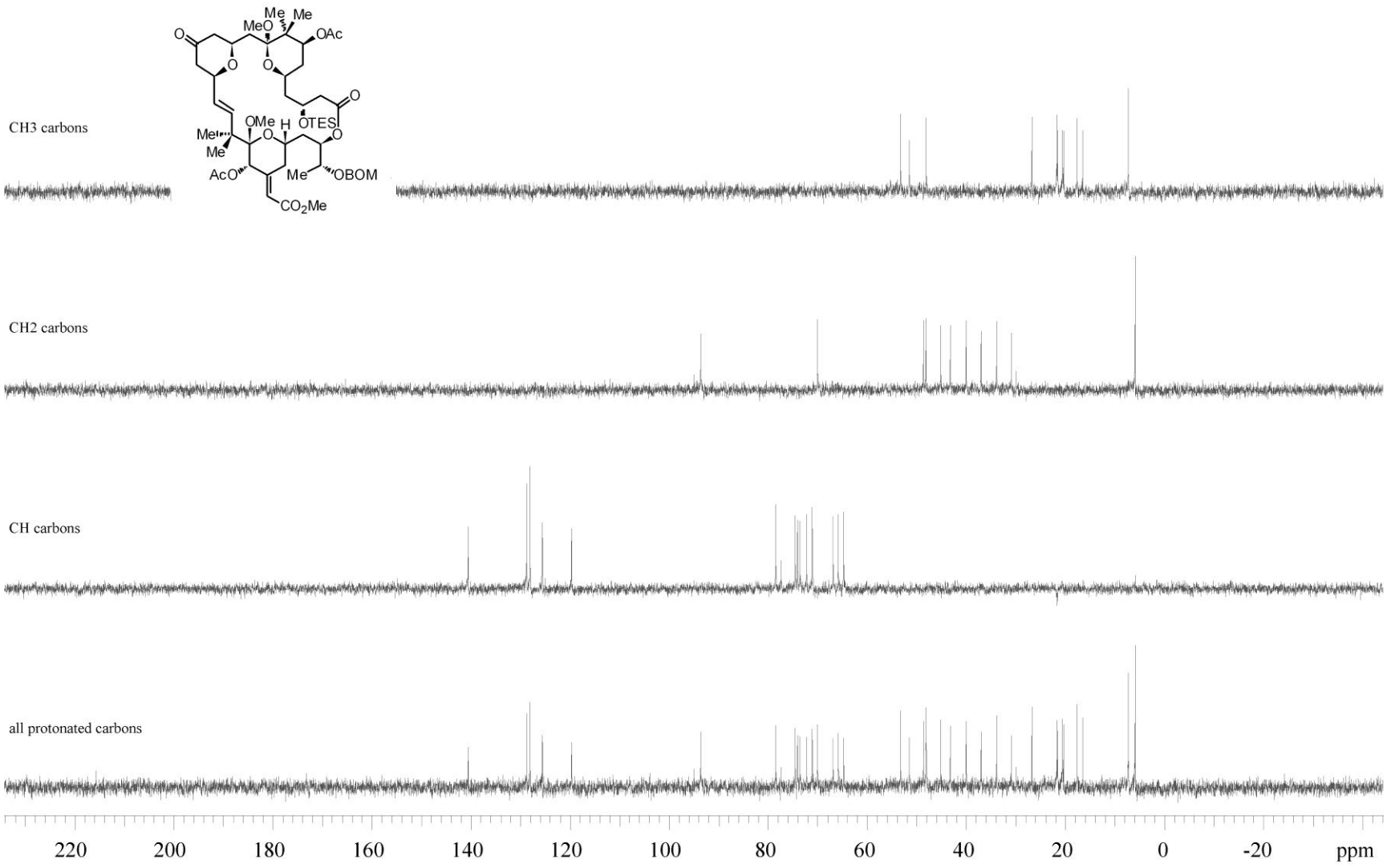

200
180
160
140
120
100
80
60
40
20
ppm

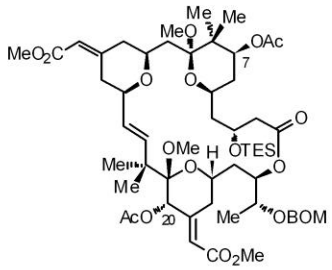

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
Me
Me
MeO
OAc
O
O
O
OMe
H
OTES
O
Me
Me
AcO
Me
OBOM
CO2Me
220
200
180
160
140
120
100
80
60
40
20
0
-20
ppm

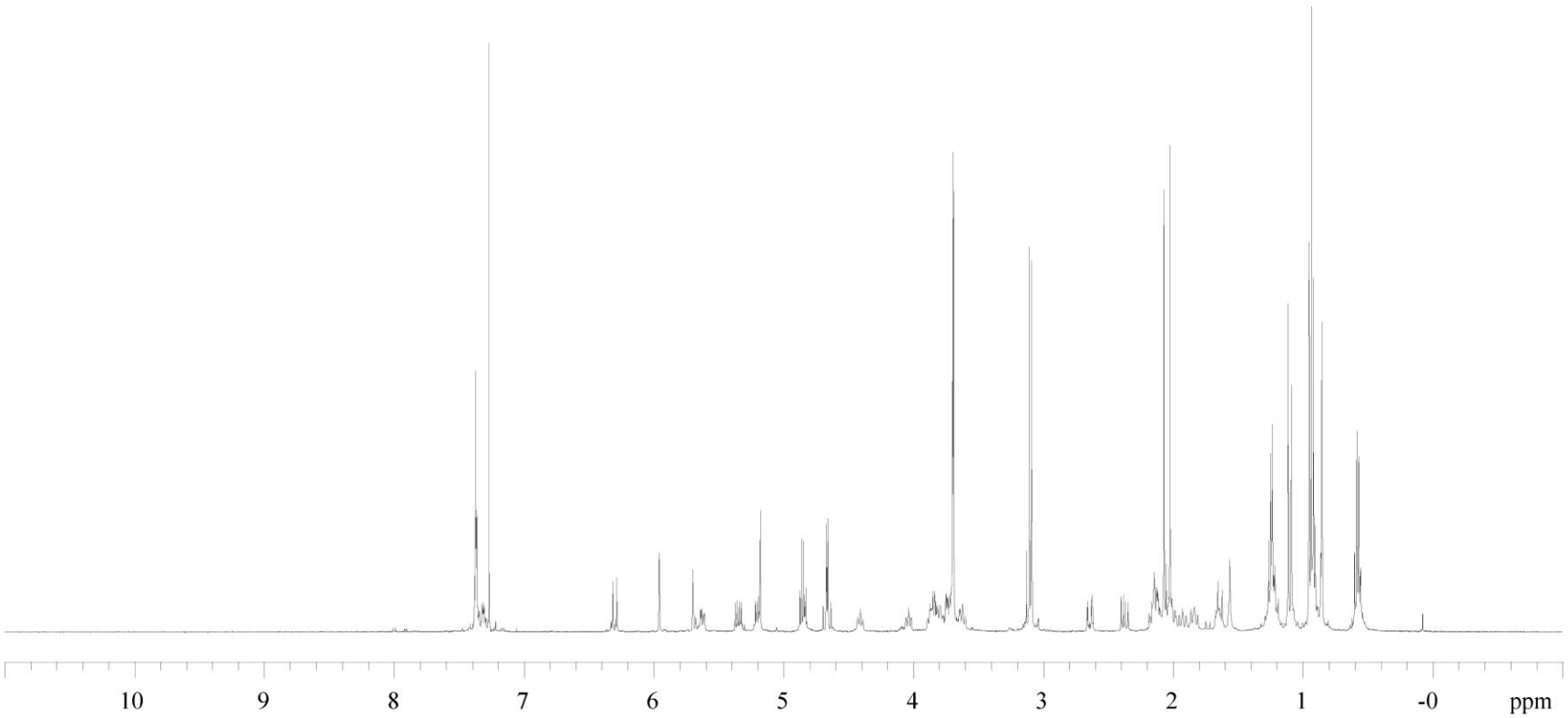

Me Me
MeO
OAc
O
O
O
O
OMe H OTES
Me
Me
O
AcO
Me
OBOM
CO2Me

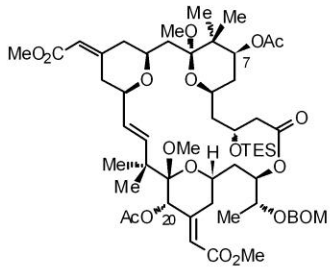
10
9
8
7
6
5
4
3
2
1
-0
ppm

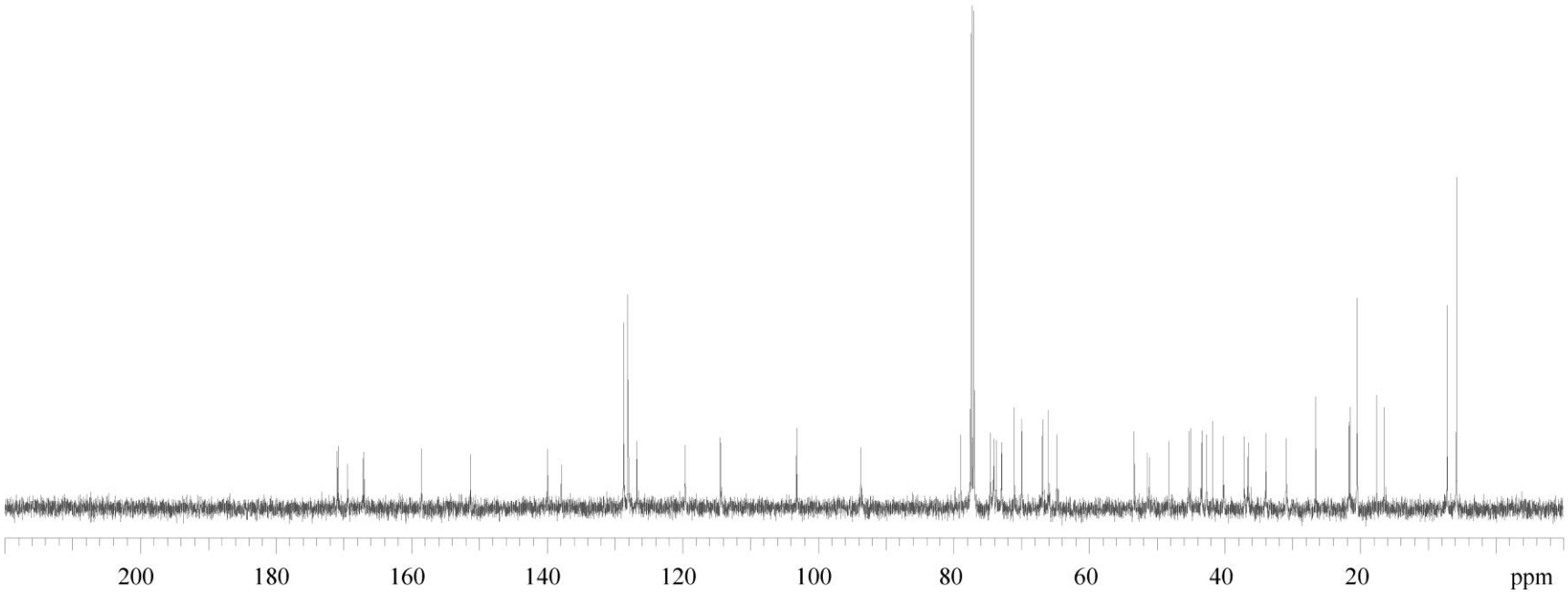

Me
Me
MeO
OAc
OMe
H
OTES
Me
Me
AcO
OBOM
Me
CO2Me
ppm
200
180
160
140
120
100
80
60
40
20

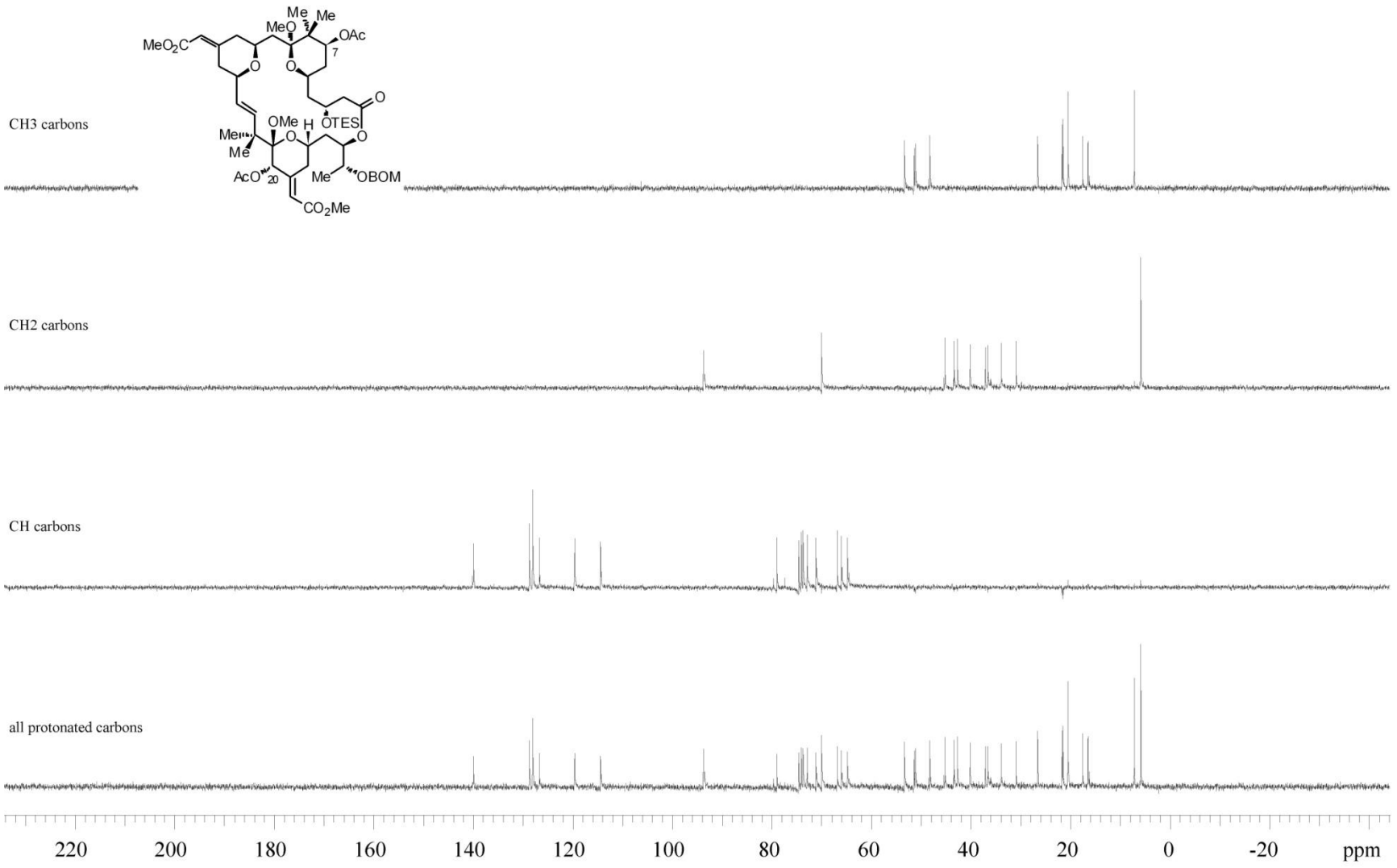

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
Me
Me
MeO
OAc
O
O
O
O
OMe
H
OTES
O
Me
Me
AcO
Me
OBOM
CO2Me
220
200
180
160
140
120
100
80
60
40
20
0
-20
ppm

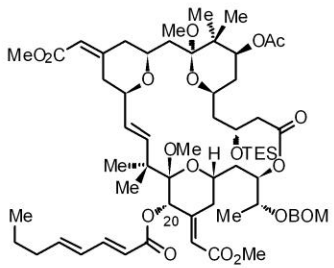

Me
Me
MeO
OAc
MeO2C
7
O
O
O
OTES
OMe
H
O
Me
Me
AcO
20
Me
OBOM
CO2Me


10
9
8
7
6
5
4
3
2
1
-0
ppm

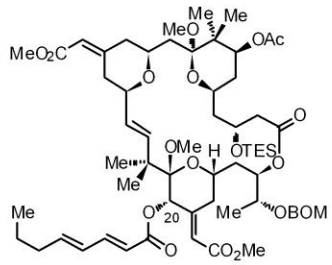

Me
Me
MeO
OAc
MeO2C
7
O
O
O
OMe
H
OTES
Me
Me
O
AcO
20
Me
OBOM
CO2Me

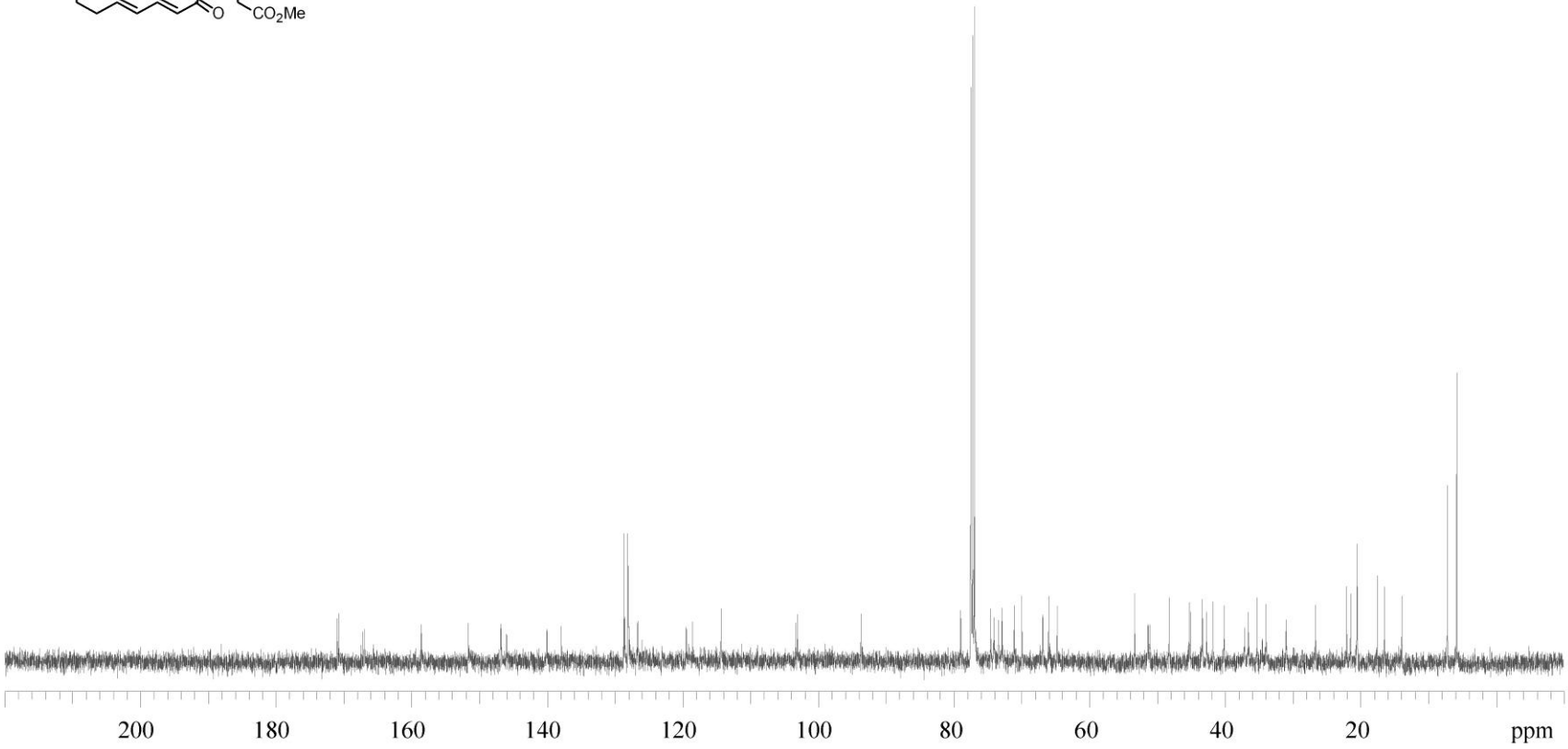

200
180
160
140
120
100
80
60
40
20
ppm

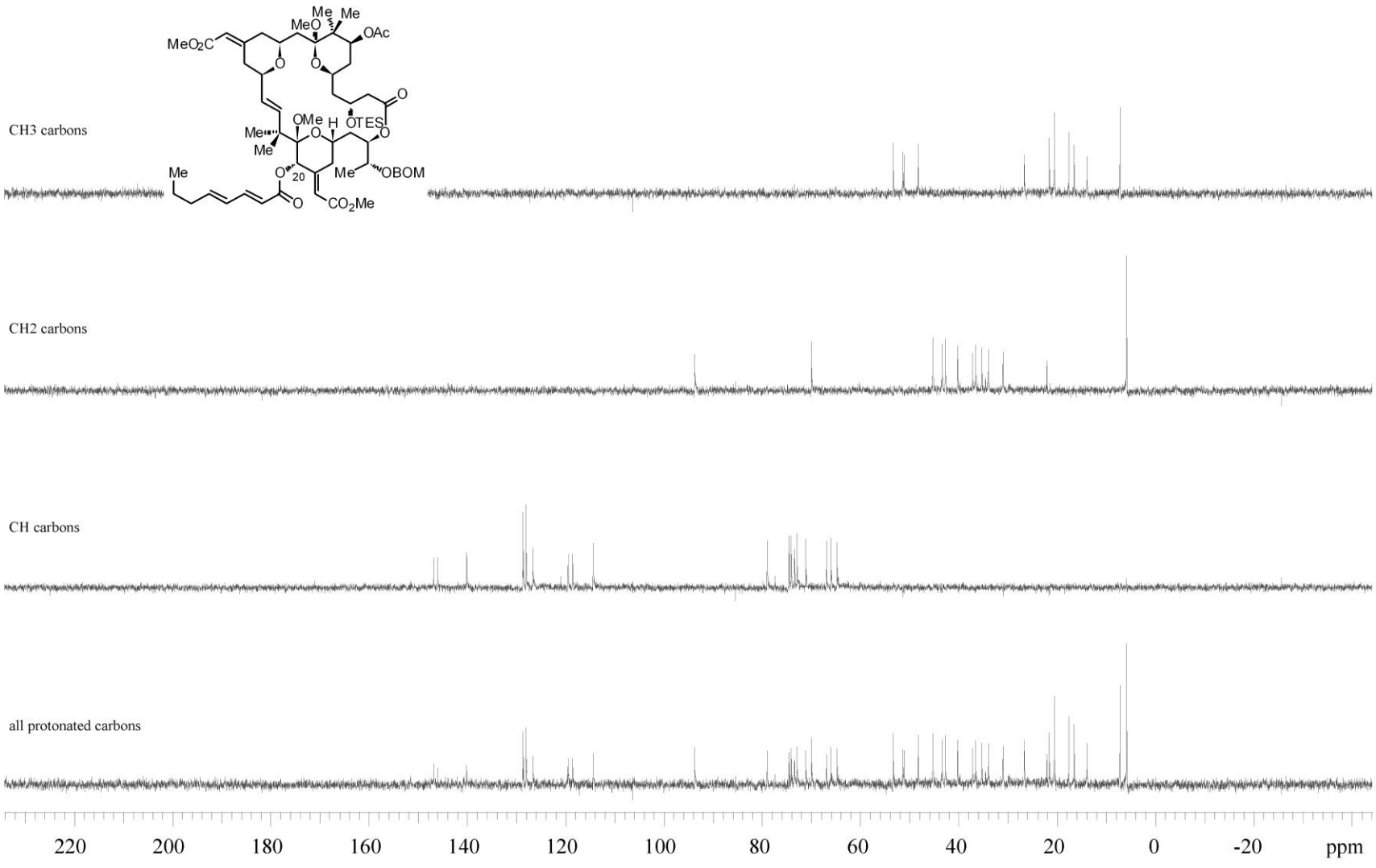

CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
220
200
180
160
140
120
100
80
60
40
20
0
-20
ppm

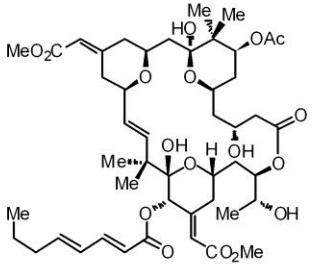

Me
MeO
Me
OAc
MeO2C
O
O
O
OMe
H
OTES
O
Me
Me
Me
O
20
Me
OBOM
O
O
CO2Me

10
9
8
7
6
5
4
3
2
1
-0
ppm

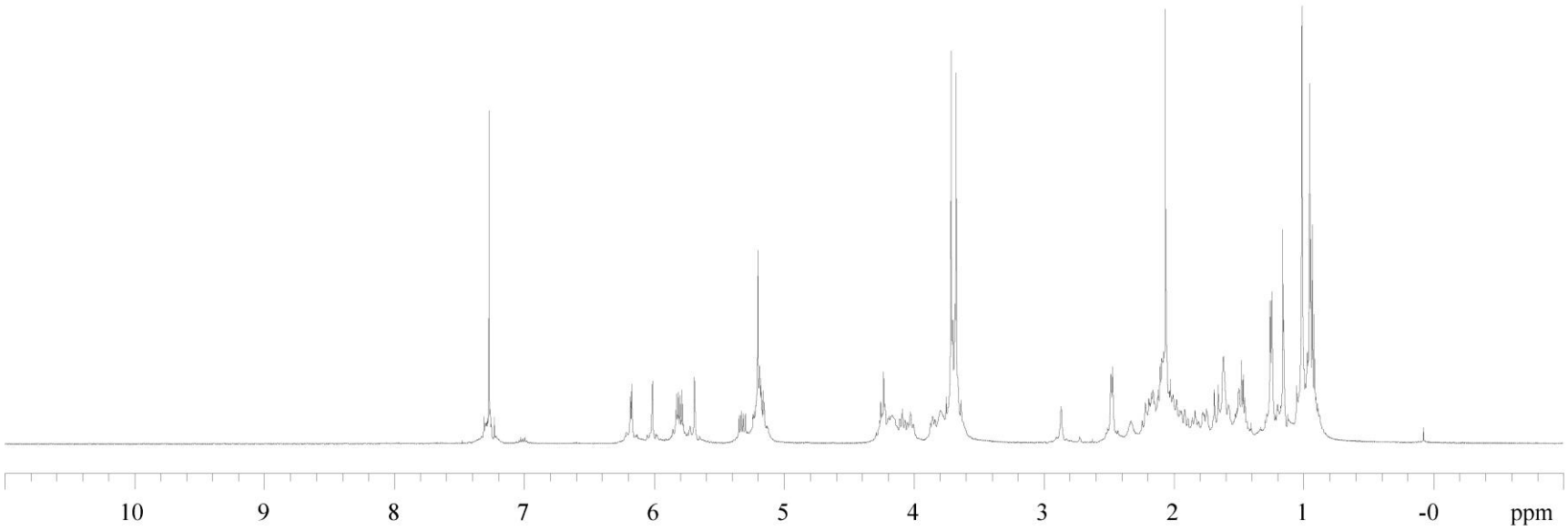

Me
Me
MeO
OAc
MeO2C
O
O
O
OMe
H
OTES
Me
Me
O
O
Me
OBOM
O
20
Me
O
CO2Me

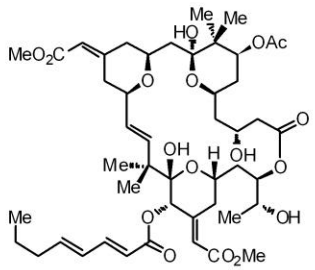

200
180
160
140
120
100
80
60
40
20
ppm


CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
220
200
180
160
140
120
100
80
60
40
20
0
-20
ppm

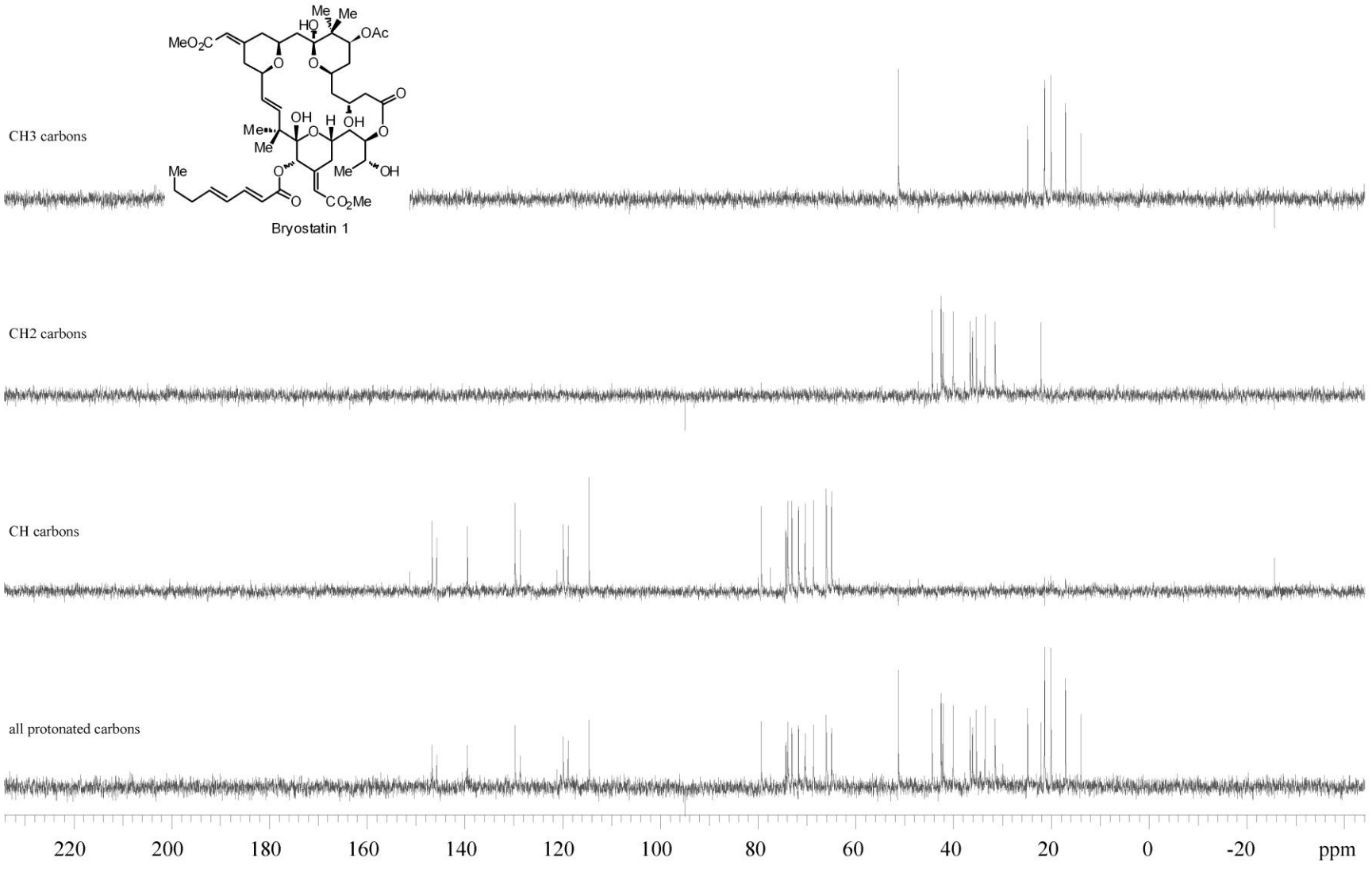


Bryostatin 1

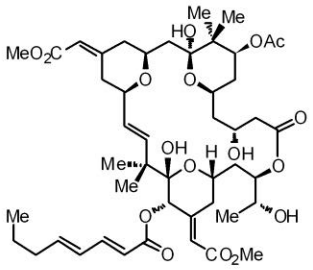




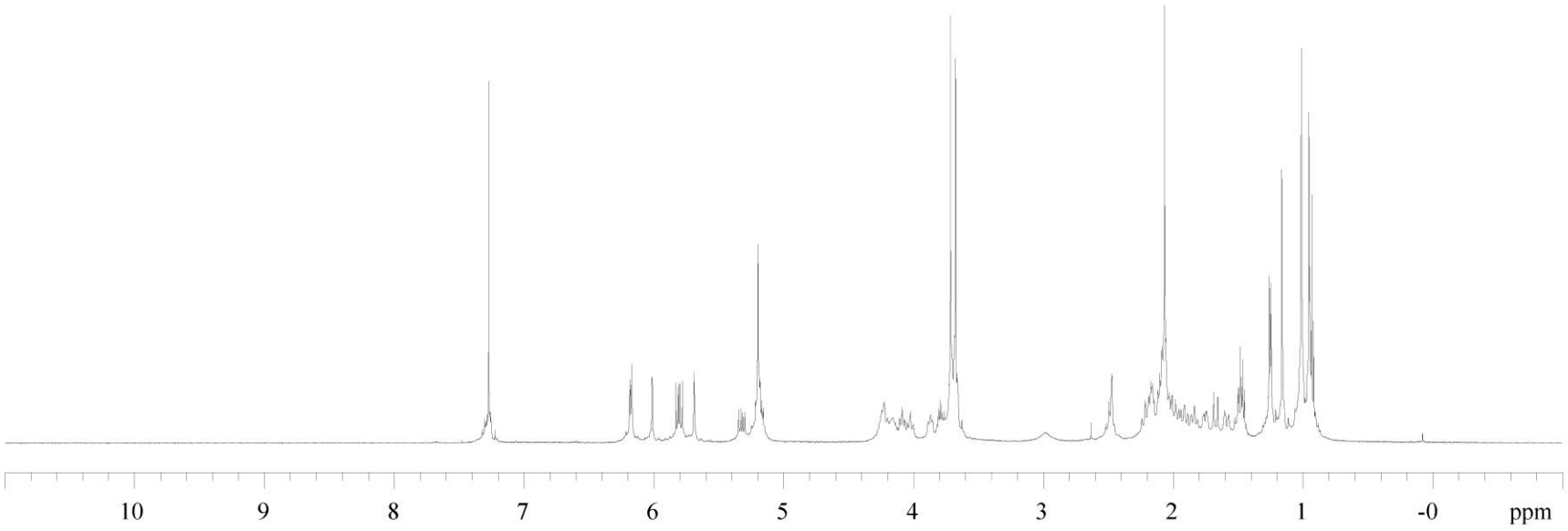
Bryostatin 1

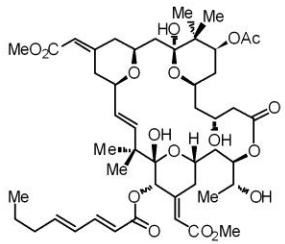


Bryostatin 1

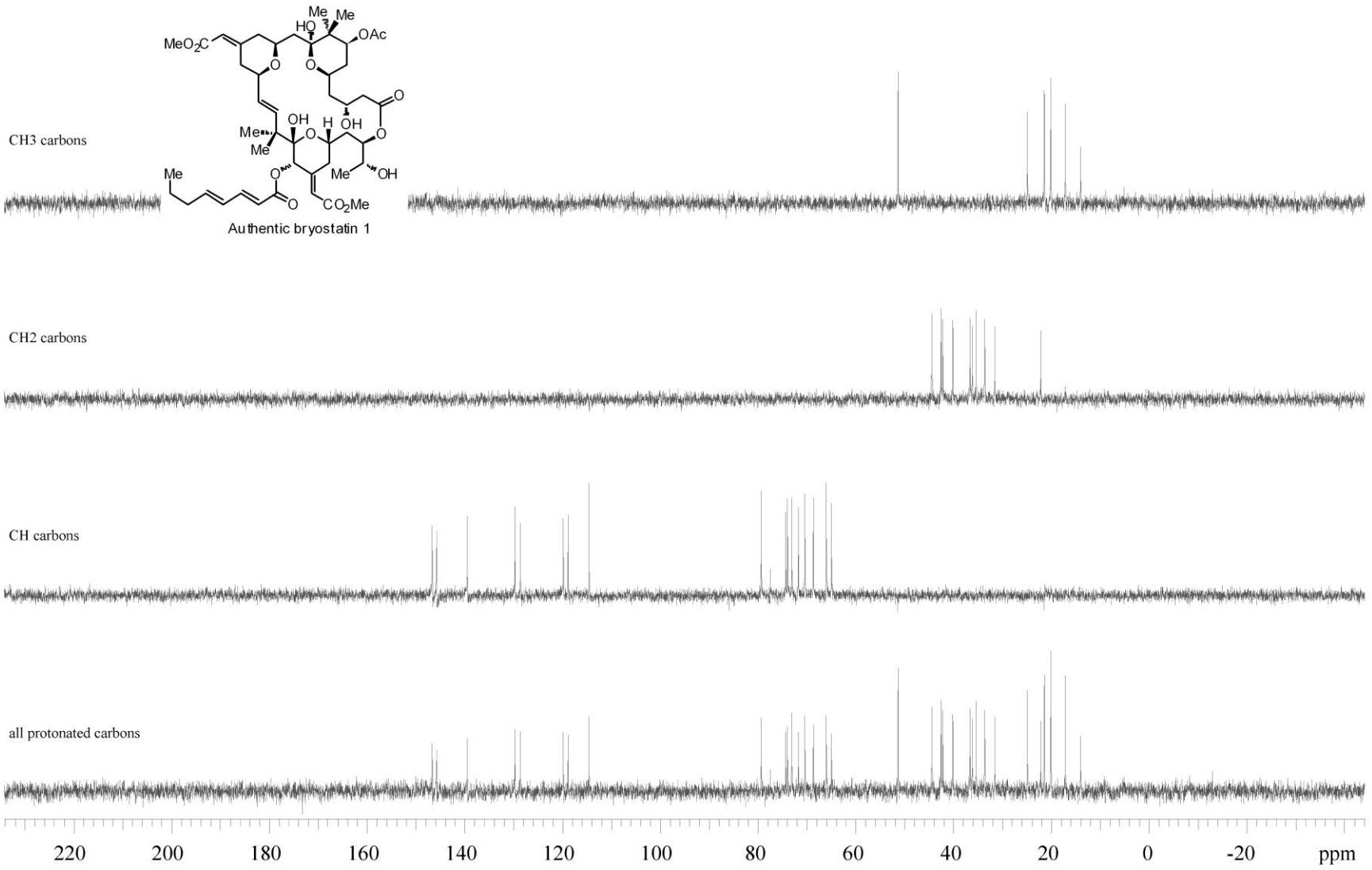


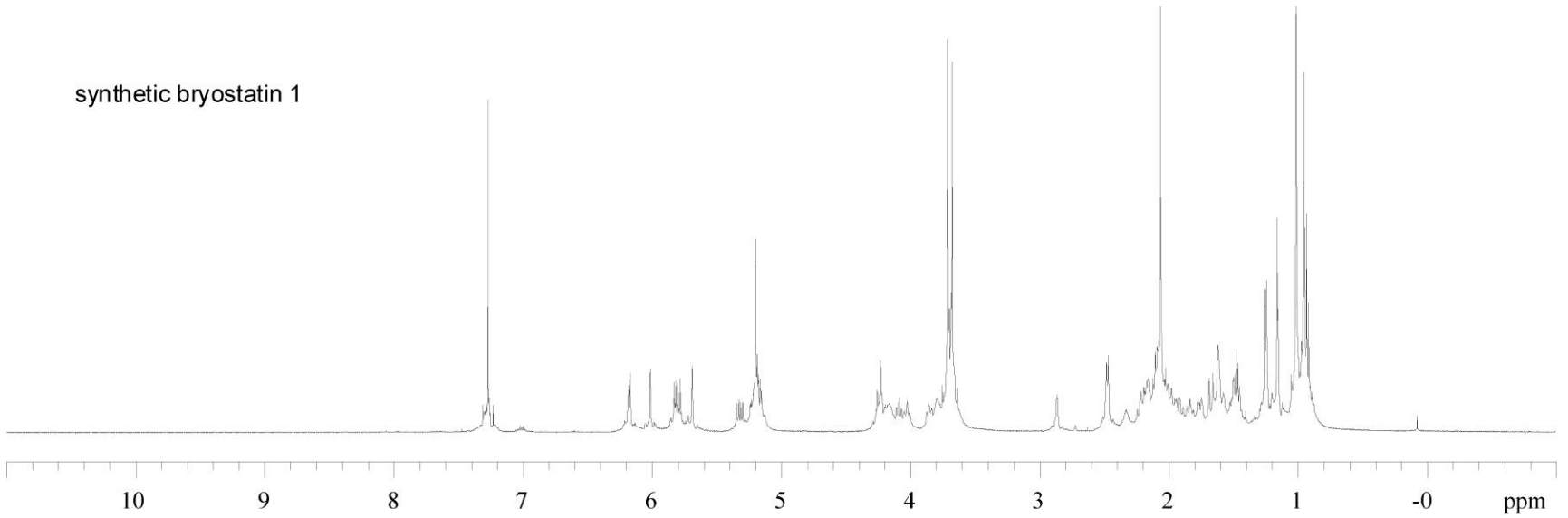
Authentic bryostatin 1

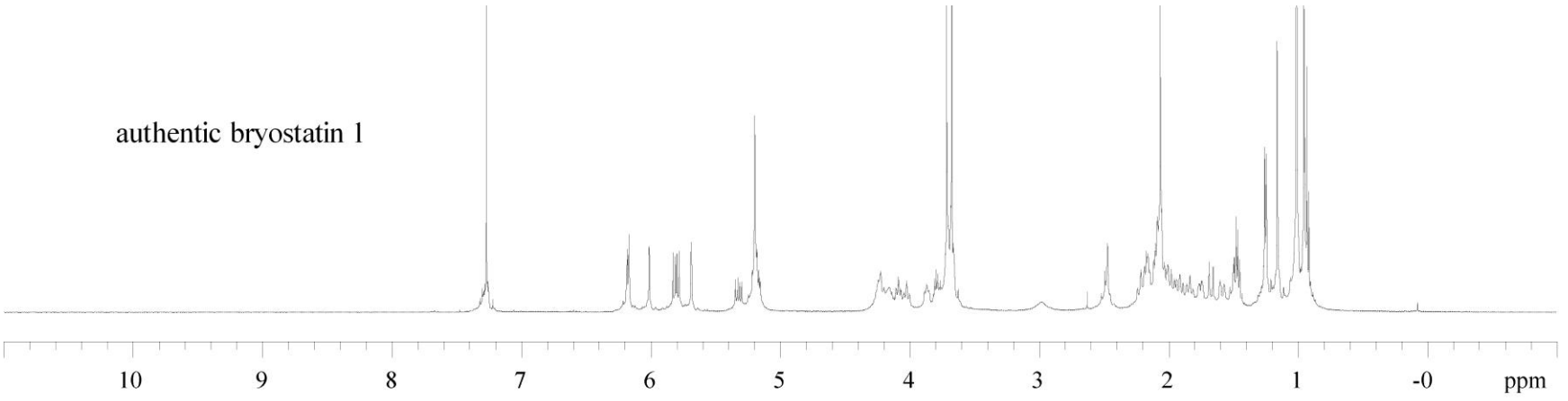


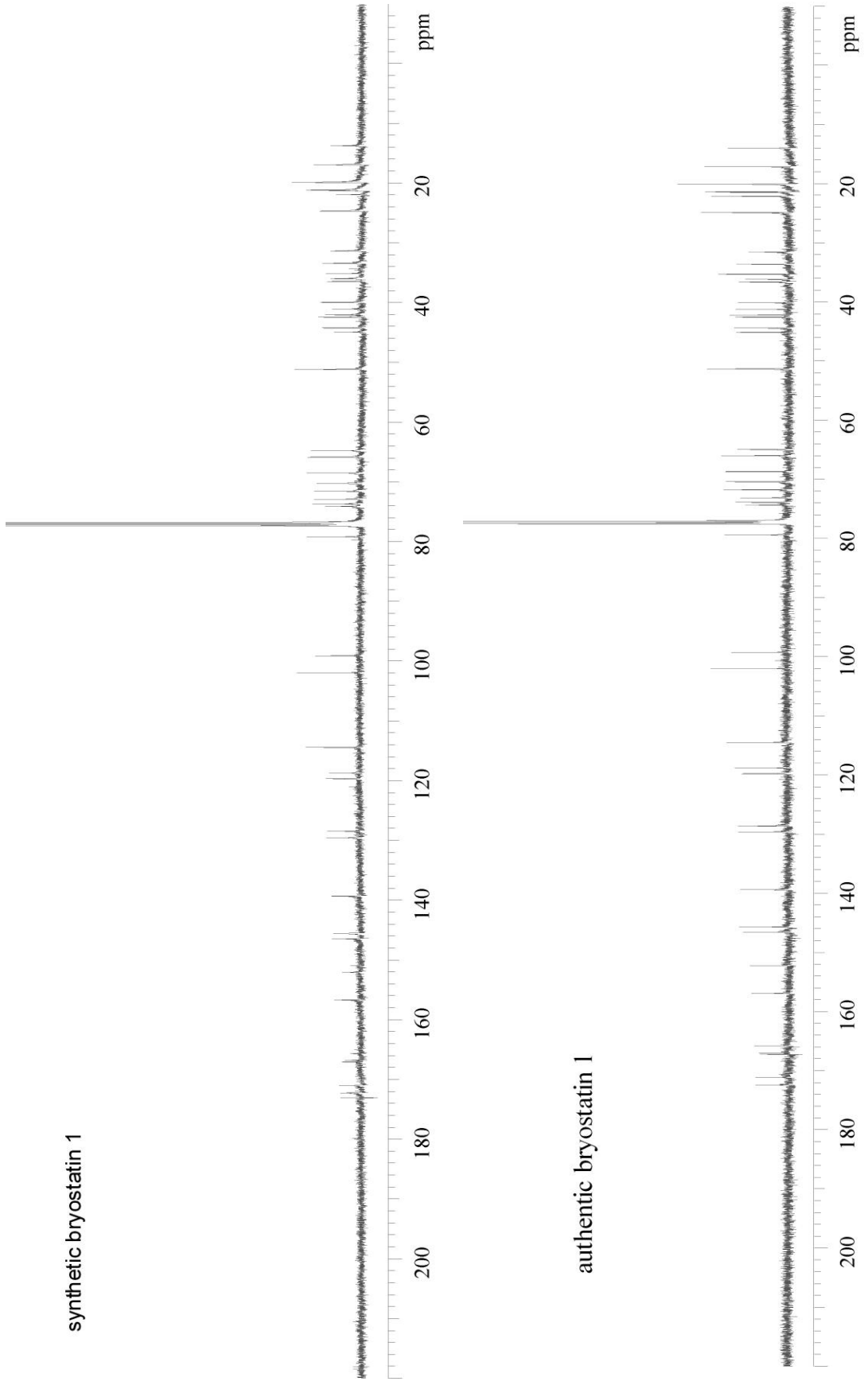


Authentic bryostatin 1


CH3 carbons
Authentic bryostatin 1
CH2 carbons
CH carbons
all protonated carbons
220
200
180
160
140
120
100
80
60
40
20
0
-20
ppm


synthetic bryostatin 1
10
9
8
7
6
5
4
3
2
1
-0
ppm


authentic bryostatin 1
10
9
8
7
6
5
4
3
2
1
-0
ppm


synthetic bryostatin 1
authentic bryostatin 1
ppm
200
180
160
140
120
100
80
60
40
20